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(54) **Method and composition for making liposomes.**

(57) Mixtures of a hydrating agent such as arginine or glutamic acid and liposome-forming materials provide a preliposome gel which spontaneously forms highly stable liposomes in aqueous solution having very high capture efficiency.

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METHOD AND COMPOSITIONS FOR MAKING LIPOSOMES

Background

This invention relates to novel liposome-forming compositions which provide a new method for forming liposomes. More specifically, this invention relates to the use of hydrating agents, compounds with at least two ionizable groups, with liposome-forming materials to make a gel which spontaneously forms liposomes when diluted with an aqueous solution.

General Description

Numerous processes and methods have been developed for making the different types and sizes of liposomes and for encapsulating active ingredient. Most of these methods have focused on the use of an organic solvent to ensure complete solubilization and uniform mixing of the phospholipids and fatty acids prior to dispersion in an aqueous system. A second development was the use of ultrasonic irradiation to disperse the phospholipid/fatty acid material.

For example Robinson, Trans. Faraday Soc., 56:1260 (1960) and Papahadjopoulos, et al. [Biochim. Biophys. Acta, 135, 639 (1967)] describe formation of phospholipid dispersions from a two-phase ether/water system involving evaporation of the ether by bubbling nitrogen through the mixture. Similarly, chloroform has been used by Chowhan, et al., Biochim. Biophys. Acta, 266:320 (1972) to insure a complete and thorough mixing of

the phospholipids prior to dispersion.

Ultrasonic dispersion, first described by D. Papahadjopoulos and N. Miller in Biochim. Biophys. Acta, 135:624 (1967) produces small unilamellar vesicles but the technique is limited because of low encapsulation efficiency.

Batzri and Korn [Biochim. Biophys. Acta, 198:1015 (1973] have used the technique of injecting the lipids in an organic phase (ethanol) into an aqueous solution. Ether was used by Deamer and Bangham in essentially the same technique [Biochim. Biophys. Acta, 443:619 (1976)].

Yet another technique involves a calcium-induced structural change in a lipid vesicle derived from or containing phosphatidylserine but is reported to have a relatively low encapsulation efficiency due to the method of reconstitution of the vesicle. See Papahadjopoulos, et al., Biochim. Biophys. Acta, 394:483 (1975) and H. Hauser, Trends in Pharm., Science 3, 274-77 (1982).

Several other patents set out methods for lipid vesicle formation of interest to this invention. U.S. patent No. 3,804,776 describes a method for producing oil and fat encapsulated amino acids for polypeptides by dispersing dry powders of the material in a molten mixture of the fat or oil and then pouring the mixture into water. The encapsulated material is contained within relatively large droplets of lipid. Such vesicles are not suitable for IV injection and are limited to use only for oral administration.

Entrapment of certain drugs in lipid vesicles by freezing the aqueous phospholipid dispersion of the drug and lipid is described in U.S. patent No. 4,016,100.

Papahadjoplous and Szoka, in U.S. patent No. 4,235,871, disclose a method for making liposomes where the vesicle-forming material is dissolved in an organic solvent and then mixed with an aqueous solution containing

the material to be encapsulated. An homogeneous water-in-oil emulsion is formed and the organic solvent is evaporated to give a gel-like mixture. This gel is then suspended in water to form the vesicles.

Another process of interest is disclosed in U.S. patent No. 3,932,657, which teaches the encapsulation of polyaminopolycarboxylic chelating agents, EDTA and TDPA. Yet another U.S. patent, 4,217,344, issued to Vanlerberghe, et al., notes that certain additives can be combined with nonionic lipid compounds so as to modify the permeability and superficial charge of lipid vesicles. Among the several additives mentioned are polypeptides and proteins. Mackaness, et al. describe in U.S. Patent 4,192,859, contrasts media containing liposomes as carriers. The list of contrast agent salts includes arginine salt, cystein salt, glycine salt, glycyl glycine salt, and N-methylglucosamine salts. These materials are characterized as aliphatic and alicyclic amines which can be used to prepare water soluble salts of the various contrast agents which may be employed in X-ray contrast agents.

An article in Science, March, 1984, by Janos Fendler makes reference to a number of synthetic surfactants which may be used in forming vesicles. Fendler references quaternary ammonium and carboxylate, sulfate, sulfonate and phosphate zwitterionic materials which are referenced to the following literature articles: J. H. Fendler, Acc. Chem. Res., 13, 7 (1980); T. Kunitake and S. Shinkai, Adv. Phis. Org. Chem., 17, 435 (1980); T. Kunitake, et. al., J. Am. Chem. Soc., 103, 5401 (1981); J. H. Fuhrhop and J. Matthieu, J. Chem. Soc. Chem. Commun. p. 141 (1983); and W. Talmon, et. al., Science, 221, 1047 (1983).

It has been discovered that liposomes are spontaneously formed when phospholipids and/or fatty acids, liposome forming materials, are dispersed in an aqueous medium containing a hydrating agent. This class

of compounds, for the purpose of this invention, is illustrated by arginine and similar amino acids which have at least one ionizable functionality at both the alpha and omega termini of the molecule. These "hydrating" compounds will have either the same type of ionizable group on the molecule, both cationic, or both anionic, or have ionizable groups of opposite charge. It isn't required that the ionizable groups be on the alpha and omega carbons but such compounds represent the preferred embodiments of this invention.

In practical terms, liposomes formed using this invention are formulated as a "pre-liposome gel" referred to herein as a "gel" where a phospholipid and/or fatty acid mixture capable of forming liposomes is mixed with an appropriate, concentrated aqueous solution of the hydrating compound. This gel, upon dispersion in an aqueous medium efficiently and spontaneously forms liposomes without solvent evaporation, input of ultrasonic irridation or any of the other means developed to insure proper formation of lipid vesicles, liposomes.

Liposomes made with hydrating agents are more stable than the ones produced by conventional methods, including those formed using organic solvents and ultrasonic energy. Liposome formulations having these hydrating agents suffer none of the solvent removal problems of the current technology nor are the liposomes beset by the non-uniform, destructive forces of ultrasonic irradiation inherent in the older methods.

Additionally, the pre-liposome gel can be denydrated and stored for a substantial period of time and still be capable of spontaneously forming liposomes upon rehydration.

The pre-liposome gel is extraordinarily stable, stable enough to be autoclaved for sterilization. Furthermore, water-soluble or water-insoluble substances to be encapsulated can be added to the gel and will then

be incorporated into the liposomes upon dispersion of the gel. This capability has the effect of greatly enhancing the encapsulation efficiency.

Furthermore, it also has been discovered that the concentration of hydrating agent influences the size of the resulting liposomes in a predictable manner at a given pH. Correspondingly, varying the pH of the dispersing aqueous solution while holding the hydrating agent constant also influences the size of the liposomes produced in a predictable manner.

Thus, the present invention provides an easier, more convenient and predictable means for controlling vesicle size over methods previously available. This method also has no limitations on the concentrations of lipids in the preparation of liposomes.

Summary of the Invention

This invention relates to a liposome product made by dispersing in an aqueous medium, in a manner adequate to form liposomes, a composition comprised of:

a. liposome-forming material;

b. a hydrating agent wherein the hydrating agent is present in a molar ratio of between 1:20 and 1:0.05 relative to the liposome-forming material;

c. water in an amount up to 300 moles relative to the solids; and

d. optionally, a material to be encapsulated.

In a second aspect, this invention covers a composition capable of forming liposomes when dispersed in an aqueous medium, which composition comprises:

a. liposome-forming material;

b. a hydrating agent wherein the hydrating agent is present in a molar ratio of between 1:20 and 1:0.05 relative to the liposome-forming material;

c. optional material to be encapsulated; and

d. water in an amount up to 300 moles relative to the

solids.

In another aspect, this invention relates to a means for forming stable liposomes wherein the means comprises adding a hydrating agent to liposome-forming materials in a molar ratio of between 1:20 and 1:0.05 relative to the liposome-forming material and dispersing the mixture in an aqueous medium in a manner adequate to form liposomes. Alternatively, the hydrating agent, liposome-forming material and the substance to be encapsulated can be added separately to the aqueous medium, then means for dispersion applied to form the liposomes.

SPECIFIC EMBODIMENTS

Definitions

For the purpose of this invention, a hydrating agent means a compound having at least two ionizable groups, preferably of opposite charge, one of which is capable of forming an easily dissociative ionic salt, which salt can complex with the ionic functionality of the liposome-forming material. The hydrating agent inherently does not form liposomes in and of itself. Such agent will also be physiologically acceptable, i.e., it will not have any untoward or deleterious physiological effect on the host to which it is administered in the context of its use in this invention.

Complexing in this context denotes the formation of dissociative ionic salts where one functionality associates with the ionic functionality of the liposome-forming material and the other functionality has hydrophilic properties which impart water-solubility to the resulting complex.

Hydrated complex means the complex formed between the hydrating agent and the liposome-forming material whereby there is formed a specific semi-crystalline arrangement of molecules. Certain particular, specific spectral data

characterized the presence of this complex.

Mixtures of the hydrating agent and liposome-forming materials with certain, discrete amounts of water form a gel-like mass. When in this gel form, the hydrating agent and the liposome-forming material arrange into a "hydrated complex" which is a highly ordered liquid crystal. While the liquid crystal structure varies with pH and amount of hydrating agent, the liquid crystal structure remains. NMR spectroscopy confirms that the crystal structure consists of multilamellar lipid bilayers and hydrophilic layers stacked together in alternating fashion. The ^{31}P-NMR spectrum exhibits an anisotropic peak, further confirming the existence of multilamellar bilayers.

The word "liposome" has been proposed and accepted as the term to be used in the scientific literature to describe synthetic, oligolamellar lipid vesicles. Such vesicles are usually comprised of one or more natural or synthetic lipid bilayers surrounding an internal aqueous phase.

The phrase "liposome-forming material" refers to all natural and synthetic compounds which have one ionizable function and a hydrophobic component, a fatty component, such as the phospholipids, non-volatile fatty acids, non-volatile alkyl amines and the like which singly or in combination form liposomes when dispersed in an aqueous medium. This definition is not intended to be limiting in its scope but is to be read to include all compounds capable of forming lipid vesicles, past, present and future.

Examples of liposome-forming materials include saponifiable and non-saponifiable lipids, e.g., the acyl glycerols, the phosphaglycerides, the sphingolipids, the glycolipids, etc. The fatty acids include saturated or unsaturated alkyl (C_8-C_{24}) carboxylic acids, mono-alkyl (C_8-C_{27}) esters of C_4-C_{10} dicarboxylic acids (e.g., cholesterol hemi-succinic acid

and fatty acid derivatives of amino acids in which any N-acyl carboxylic acids also are included (e.g., N-oleoyl threonine, N-linoleoyl serine, etc.). Mono- or di-alkyl (C_8~C_{24}) sulfonate esters and mono- or di-alkyl (C_8~C_{24}) phosphate esters can be substituted for the fatty acids. Furthermore, mono- or di-acyl (C_8~C_{24}) glycerol derivatives of phosphoric acids and mono- or di-acyl (C_8~C_{24}) glycerol derivatives of sulfuric acids can be used in place of the fatty acids.

Additionally, the fatty acids also can be replaced by saturated or unsaturated alkyl amines (e.g., C_8~C_{24} NH_2), C_8~C_{24} fatty acid derivatives of amines (e.g., C_8~C_{24} $CONH$~NH_2), C_8~C_{24} fatty alcohol derivatives of amino acids (e.g., C_8~C_{24} OOC~NH_2), and C_8~C_{24} fatty acid esters of amines (e.g., C_8~$C_{24}COO$~NH_2).

Photopolymerizable lipids and/or fatty acids (or amines) (e.g., diacetylenic fatty acids) also can be included, which can provide a sealed liposome with cross-linked membrane bilayers upon photo-initiation of polymerization.

Although the primary components of these liposomes will be lipids, phospholipids, other fatty acids, there may also be added various other components to modify the liposomes' permeability. There may be added, for example, non-ionic lipid components such as polyoxy alcohol compounds, polyglyceral compounds or esters of polyoles, polyoxyalcolinolated alcohols; the esters of polyoles and synthetic lipolipids, such as cerebrosides. Other materials, such as long chain alcohols and diols, sterols, long chain amines and their quaternary ammonium derivatives; polyoxyethylenated fatty amines, esters of long chain amino alcohols and their salts and quaternary ammonium derivatives; phosphoric esters of fatty alcohols. polypeptides and proteins.

The composition of the liposome can be made of more

than one component of the various kinds of lipids, the fatty acids, alkyl amines, or the like, and the hydrating agents.

It also has been discovered that the lipid composition may not require the inclusion of the fatty acids (or the amines) or the hydrating agents to form the "pre-liposome gel" or liposomes, if the lipid component itself or the substances (e.g., medicaments, biologically active compounds, cosmetics, etc.) to be encapsulated possess the aforementioned properties. For example, the mixture of dipalmitoylphosphatidylcholine (DPPC) and distearoyl phosphatidylethanolamine forms the "pre-liposome gel" or liposomes with aqueous glutamic acid solution and the mixture of DPPC and oleic acid with aqueous epinephrine solution forms the "pre-liposome gel" and liposomes.

For pharmaceutical application as a liposome drug delivery system, however, the composition of phospholipids, oleic acid (or phosphatidylethanolamine) and arginine or lysine (or glutamic acid and/or aspartic acid) was preferred.

When solids are referred to, the liposome-forming materials, hydrating agents, and material to be encapsulated if any, is what is being referred to.

Preferred Embodiments

The preferred hydrating agents of this invention are alpha amino acids having an ionizable omega subsitution such as a carboxylate, amino, and guanidino function and those compounds represented by the formula:

$$X-(CH_2)n-Y \quad I$$

wherein

X is $H_2N-C(NH)-NH-$, H_2N-, ZO_3S-, Z_2O_3P-, or ZO_2C- wherein Z is H or an inorganic or organic cation;

Y is $-CH(NH_2)-CO_2H$, $-NH_2$, $-NH-C(NH)-NH_2$, $-COOH$, $CH(NH_2)SO_3Z$ or $ZH(NH_2)PO_3Z_2$ wherein Z is defined above; and

n is the integer 1-10; or

a pharmaceutically acceptable salt thereof. Also included in the list of preferred compounds are the N,N'-dialkyl substituted arginine compounds and similar compounds where the alkyl chain length is varied.

More preferred hydrating agents are the omega-substituted, alpha amino acids such as arginine, its N-acyl derivatives, homoarginine, gamma-aminobutyric acid, asparagine, lysine, ornithine, glutamic acid, aspartic acid or a compound represented by the following formulae:

$$H_2NC(NH)-NH-(CH_2)_n-CH(NH_2)COOH \quad II$$

$$H_2N-(CH_2)_n-CH(NH_2)COOH \quad III$$

$$H_2N-(CH_2)_n-NH_2 \quad IV$$

$$H_2NC(NH)-NH-(CH_2)_n-NH-CH(NH)-NH_2 \quad V$$

$$HOOC-(CH_2)_n-CH(NH_2)COOH \quad VI$$

$$HOOC-(CH_2)_n-COOH \quad VII$$

$$HO_3S-(CH_2)_n-CH(NH_2)COOH \quad VIII$$

$$H_2O_3S-(CH_2)_n-CH(NH_2)COOH \quad IX$$

$$HO_3S-(CH_2)_n-CH(NH_2)SO_3H \quad X$$

$$H_2O_3S-(CH_2)_n-CH(NH_2)PO_3H_2 \quad XI$$

wherein n is 2-4.

The most preferred compounds are arginine,

homoarginine, gamma-aminobutyric acid, lysine, ornithine, glutamic acid or aspartic acid.

About 1:20 molar ratio of hydrating agent relative to the liposome-forming material will provide the salutory effects of this invention with an upper limit of about 1:0.05. The preferred concentration range for the hydrating agent is between a 1:2 to 1:0.5 molar ratio of the hydrating relative to the liposome-forming material.

The hydrating agents of this invention may be used alone or as a mixture. No limitation is implied or intended in the use of mixtures of these hydrating materials.

As a practical matter, thus a matter of preference, if liposomes are prepared with liposome-forming materials having a negative charge, it is preferred to use hydrating agents which contain at least one ionizable nitrogen, such as arginine, homoarginine, lysine, ornithine, and the like. Conversely, if the amphipatic materials used to form the liposomes are nitrogen-based, it is preferred to use a di-acid such as glutamic acid, aspartic acid; any of the alkyl di-acids such as the simple di-acids such as valeric acid, caprylic, caproic, capric or the like; or those di-acids having two phosphate, or sulfate functionalities; or those di-acids having mixed $-COOH/-SO_3H$ or $-COOH/-PO_3H_2$ functions.

Source of hydrating agents

The hydrating agents of this invention are listed in the catalogue of many chemical producers, can be custom manufactured by such producers, or can be made by means known in the art.

Arginine, homoarginine, lysine, glutamic acid, aspartic acid, and other naturally occurring amino acids may be obtained by the hydrolysis of protein and separation of the individual amino acids or from bacterial sources.

The compounds of formula II can be made by the method of Eisele, K. et al, Justusliebigs. Ann. Chem., p 2033 (1975). Further information on several representative examples of these compounds is available through their respective Chemical Abstracts Service numbers as follows: norarginine, CAS # 14191-90-3; arginine, CAS # 74-79-3; and homoarginine, CAS # 151-86-5.

For representative examples of formula III, see for 2,4-diaminobutanoic acid CAS # 305-62-4 and for lysine CAS # 56-87-1.

Methods for making representative compounds of formula IV are available from Chemical Abstracts as follows: ethane diamine, CAS # 305-62-4; propane diamine - 54618-94-9; and 1,4-diaminobutane, CAS # 52165-57-8. See specifically Johnson, T.B., J. Am. Chem. Soc., 38, 1854 (1916).

Of the compounds of formula VI, glutamic acid is well known in the art and is available from many commercial sources. How to make other representative compounds is contained in the literature, for example:
2-aminohexandioic acid - CAS # 62787-49-9 and
2-aminoheptandioic acid - CAS # 32224-51-0.

Glutamic acid, the compound of formula VII where n is 2 is well known in the art and can be made by the method of Maryel and Tuley, Org. Syn, 5, 69 (1925). Other representative compounds in this group can be made according to the art as referenced by the following CAS numbers: hexadioic acid, CAS # 123-04-9 and heptadioic acid, CAS # 111-16-0.

Homocysteic acid is known in the art referenced by CAS # 56892-03-6. The compound 3-sulfovaline is described in the literature referenced by CAS # 23405-34-2.

Pre-liposome Gel

Mixtures of liposome-forming materials and one or more hydrating agents with up to 300 moles of water

relative to the total liposome-forming material gives a gel which forms liposomes directly therefrom upon addition of an aqueous medium. This gel is labeled a pre-liposome gel because i.) of its structural characteristics which are essentially those of liposomes and, ii.) the gel's facility for being converted into liposomes upon dilution with an aqueous medium. Aqueous medium in excess of about 300 moles cause the beginning of liposome formation.

The structure of this gel is a highly ordered liquid crystal which forms an optically clear solution. The X, Y, and Z dimensions of the liquid crystal vary with the concentrations of hydrating agent at the constant pH as well as with the pH of the solution. By varying the hydrating agent concentration at constant pH or changing the pH while maintaining percentage of hydrating agent, the size and number of lamellae structures of the lipid bilayers of the subsequent liposome vesicles can be controlled.

The gel structure itself can accommodate up to approximately 300 moles of water per mole of lipid or fatty acid without disturbing the stability of the gel structure. The structure of the gel as determined by proton NMR spectroscopy is comprised of multilamellar lipid bilayers and hydrophilic layers stacked together in an alternating fashion. The ^{31}P-NMR spectrum of the same gel exhibits an anisotropic peak further confirming that the gel consists of a multilamellar bilayer.

This gel can be autoclaved, a convenient means of sterilization. Furthermore, the gel shows no discoloration and remains clear at room temperature for at least one year after being autoclaved. The gel can further be sterilized by filtration through an appropriate sterilization filter.

Upon dispersion of the gel into an aqueous medium, liposomes are efficiently and spontaneously produced.

The pre-liposome gel, with or without the material to be encapsulated, also can be dehydrated (lyophilized) and the powder rehydrated to form liposomes spontaneously, even after a long period of storage. This capability makes the invention particularly useful for administering water-sensitive medicaments where long term pre-use storage is needed.

Either water insoluble or water soluble chemicals, drugs, cosmetics, food materials and the like, can be incorporated into liposomes prepared using this material and by this method. Accordingly, the gel may be used as a delivery system for the administration of medicaments via oral, parenteral, topical, intravenous, suppository routes or any other route of drug or chemical delivery.

The use of these liposomes is not limited to human or mammalian use but can be used in any industrial, agricultural, pharmaceutical, cosmetic or chemical application where lipid vesicle encapsulation and administration of compounds or materials is warranted or useful.

The versatility of the present invention is illustrated, but not limited, by the following examples.

Example #1

Preparation of Liposomes (Gel Phase)

Dipalmitoylphosphatidylcholine, 3.0 grams, was weighed into a 50 ml beaker. Oleic acid 1.2 grams was added and mixed together to form a uniform paste.

Arginine 0.72 grams in 30 ml of distilled deionized water was added to the dipalmitoylphosphatidylcholine-oleic acid paste and heated to 45°C. With mixing by hand, the mixture formed a clear stable gel. The gel was stored and liposomes later formed by diluting the gel with phosphate buffered saline.

Example #2

Preparation of Liposomes

Dipalmitoylphosphatidylcholine, 120 mg, and 24 mg of oleic acid were added together and mixed thoroughly until a white homogeneous paste was observed.

Then 20 mg of arginine was dissolved into 60 ml of phosphate buffered saline (ionic strength = 0.15, pH = 7.4). The arginine-saline solution was added to the paste and heated to 40°C for 1/2 hour, or until a slightly turbid solution was observed.

Example #3

Large Scale Gel and Liposome Preparation

i). Gel Manufacture: To 50 grams of egg phosphatide powder type 20 (Ashai ChemicalS) was added 20 grams of oleic acid N.F. Mixing gave a white paste which was cooled to 4°C and ground into a fine powder. This powder was added to an aqueous solution containing 20 grams of arginine and 500 grams of distilled deionized water. The mixture was mixed with a spatula as the solution was heated to about 35°C to help hydrate phospholipids. A homogeneous, slightly yellow gel was formed. This gel can be stored at 4°C or can be frozen and later reconstituted.

ii). Manufacture of Liposomes: The gel prepared in the preceding paragraph was taken from cold storage and returned to room temperature. It was then mixed with 2 liters of phosphate buffered saline, pH 7.4. A white opaque liposome solution was formed.

Example #4

Liposome Formation from the Gel

A homogeneous paste of 1.0 gram of dipalmitoylphosphatidylcholine (DPPC) and 400 mg of oleic acid was formed. Then 300 mg of arginine was mixed in 10 ml of phosphate buffered saline, heated to 45°C and

added to the DPPC/oleic acid paste to form liposomes.

Example #5

Pre-Liposome Gel

One gram of dipalmitoylphosphatidylcholine (DPPL) was mixed with 400 mg of oleic acid to form a homogeneous paste. 300 mg of arginine was mixed with 2 ml of water at 45°C until dissolved. The arginine solution was mixed with the DPPC/oleic acid paste at about 45°C to give a thick gel. Liposomes formed when this gel was diluted with phosphate buffered saline.

Example #6

Cholesterol Containing Liposomes

Cholesterol, 15 mg, was mixed with 100 mg dipalmitoylphosphatidylcholine (DPPC) to form a homogeneous powder. Then 23 mg of oleic acid was added to the powder and thoroughly mixed to form a homogeneous paste. To make liposomes, 30 mg of arginine was added to 10 ml of phosphate buffered saline, heated to 40°C and added to the DPPC/cholesterol/oleic acid paste. The combination was mixed at 40°C to obtain liposomes.

Example #7

Palmitic Acid-Containing Liposomes

Dipalmitoylphosphatidylcholine (DPPC) 250 mg was mixed with 25 mg of palmitic acid to form a uniform powder. Then 80 mg of oleic acid was mixed with this powder and heated to 45°C with constant stirring until a uniform paste was formed. Arginine 100 mg was dissolved in 25 ml of distilled deionized water and heated to 45°C. This arginine solution was added to the paste at 45°C and mixed until a uniform homogeneous gel was formed. The gel was diluted ten fold with phosphate buffered saline to form liposomes.

Example #8

Isostearic Acid-Containing Liposomes

Dipalmitoylphosphatidylcholine 100 mg was mixed with 50 mg of isostearic acid to form a uniform homogeneous paste. An arginine solution of 50 mg of arginine in 2.0 ml of distilled deionized water was made and added to the isostearic acid paste and heated to 45°C. The mixture was mixed until a clear gel was formed. Liposomes are formed upon dilution with phosphate-buffered saline.

Example #9

Oleoyl Threonine Containing Liposomes

Dipalmitoylphosphatidylcholine 125 mg and 75 mg of oleoyl threonine were added together and heated to 40° C to form a paste. Then 2 ml of distilled deionized water was added with constant mixing at 40°C. A clear gel was formed which can be diluted with phosphate buffer saline at pH 5 to form liposomes.

Example #10

Myristyl Amine Containing Liposomes

Dipalmitoylphosphatidylcholine 192 mg was added to 72 mg of myristyl amine and heated with constant mixing until a uniform paste was formed. Glutamic acid 65 mg in 5 ml of distilled deionized water was added to the paste and heated until a gel was formed. Phosphate buffered saline was added to the gel to form liposomes.

Example #11

DLPC Containing Liposomes

Dilaurylphosphatidylcholine (DLPC) 50 mg was mixed with 20 mg oleic acid to form a homogeneous paste. Arginine 20 mg was added to 10 ml of phosphate buffered saline, added to the paste and hand mixed until a turbid liposome solution formed.

Example # 12

Phosphatidylethanolamine-Glutamic Acid Liposomes

L-glutamic acid 32 mg was dissolved in 2.0 ml of distilled deionized water and the pH adjusted to 5.2 with 1.0 N sodium hydroxide. This solution was heated to 60°C, and 100 mg of phosphatidylethanolamine added. The solution was kept at 60°C with constant mixing until a uniform viscous gel was observed.

The phosphatidylethanolamine-glutamic acid gel was diluted 1/10 by phosphate buffered saline. Vesicular like structures are observed under phase contrast light microscopy.

Example #13

Dipivalylepinephrine Liposomes

One gram of dipalmitoylphosphatidylcholine was mixed with 396 mg of oleic acid, until a homogeneous paste formed. Then 400 mg of arginine in 20 ml of distilled deionized water was added to form a pre-vesicle clear gel.

To make the liposomes, 242 mg of dipivalylepinephrine was dissolved in 10 ml of distilled deionized water. Then 5 grams of the pre-vesicle gel was mixed with 5 grams of the dipivalylepinephrine solution after which 50 ml of phosphate buffered saline was added forming a liposome solution.

Example #14

Flurbiprofen Liposomes

To make these liposomes, 980 mg of dipalmitoylphosphatidylcholine, 370 mg of oleic acid, and 320 mg of flurbiprofen (free acid) were mixed together until a homogeneous paste was observed. Then 510 mg of arginine in 10 ml of purified water was added to the paste and heated to 41°C with constant mixing for 30 minutes. A clear pre-vesicle gel formed of which 5 grams was introduced into 50 ml of phosphate buffered saline and

mixed with a stir bar until a bluish translucent solution was observed.

Example #15

Levobunolol Liposomes

Thirty mg of dipalmitoylphosphatidylcholine and 15 mg of cholesterol were weighed into a 4 ml vial. Ten mg of linoleic acid was added and mixed together to form a uniform paste. Two ml of a 1% aqueous levobunolol solution containing 10 mg of arginine was added to the paste and mixed together. Then 10 ml of phosphate buffer solution was added and heated to 45°C to form liposomes.

Example #16

Pilocarpine Liposomes

To 120 mg of dipalmitoylphosphatidylcholine was added 40 mg of oleic acid to form a homogeneous paste. Forty mg of pilocarpine free base was added to 10 ml of distilled deionized water. This solution was added to the paste and heated to 45°C to form a pre-liposome gel. The resulting gel was diluted with 20 ml of phosphate buffered saline to form liposomes.

Example #17

Epinephrine Liposomes

Dipalmitoylphosphatidylcholine 250 mg was mixed with 100 mg of oleic acid to form a homogeneous paste. 50 mg of epinephrine, free base, was dissolved in 5.0 ml of distilled deionized water, heated to 40°C and added to the dipalmitoylphosphatidylcholine/oleic acid paste. This solution was mixed until a homogeneous viscous creamy gel was observed. This gel was diluted 1/5 with phosphate buffered saline (pH 7.22) to form liposomes.

EXAMPLE #18

Effect of Arginine Concentration on Liposome Size

To 502 mg of dipalmitoylphosphatidylcholine (DPPC) was added 10 microliters of (2-palmitoyl-1-C^{14}) (0.1 mCi/ml) dipalmitoylphosphatidylcholine. Chloroform was added to effect complete mixing of the radioactivity and then evaporated. Oleic acid (OA), 195 mg, was then mixed into the lipid to form a paste. Five ml of distilled water containing 119 mg of arginine was added and mixed at 45°C to form a clear gel.

One gram of the gel was weighed into four different vials and arginine was added as follows:

Sample ID	Sample Composition DPPC:OA:Arg
Vial 1 + 1 ml water	(1:1:1)
Vial 2 + 1 ml of 50 mg/ml Arg in H_2O	(1:1:3)
Vial 3 + 1 ml of 84 mg/ml Arg in H_2O	(1:1:5)
Vial 4 + 1 ml of 192 mg/ml Arg in H_2O	(1:1:10)

One-half gram of each solution was diluted in 50 ml of phosphate buffered saline of pH 7.8.

The estimated weight diameter was obtained from a Sephracyl S-1000 column chromatographic analysis employing ^{14}C-isotope labelled DPPC. The effects are given in the following Table.

Table II

Effects of Arginine Concentration on Vesicle Size

System	pH	Estimated Weight Diameter (nm)
DPPC:OA:Arg (1 :1: 1)	7.8	~220
DPPC:OA:Arg (1 :1: 3)	7.8	~140
DPPC:OA:Arg (1 :1: 5)	7.8	~90
DPPC:OA:Arg (1 :1: 10)	7.8	~20

EXAMPLE #19

pH Effect on Vesicle Size

Additionally, the vesicle size can be varied by varying the pH of the aqueous buffer solution.

To 100 mg of dipalmitoylphosphatidylcholine (DPPC) was added 25 microliters of (2-palmitoyl-1-C^{14}) (0.1 mCi/ml) dipalmitoylphosphatidylcholine. Chloroform was added to effect complete mixing of the radioactivity and then evaporated. Oleic acid (OA), 40.1 mg, was then mixed into the lipid to form a paste. One ml of a solution containing 24 mg/ml arginine in water was added to the lipid mixture and mixed at 45°C to form a clear gel.

Two 100 mg aliquots of this gel were diluted in 10 ml of phosphate buffer at pH 9.0 and 7.4 respectively.

Again, the estimated weight diameter (A) was obtained from the Sephracyl S-1000 column chromatographic analysis employing ^{14}C-isotope labelled dipalmitoylphosphotidyl-choline. Results are given in the following Table.

Table III
pH Effects on Vesicle Size

System	pH	Estimated Weight Diameter (nm)
DPPC:OA:Arg (1 :1: 1)	7.4	~>300
DPPC:OA:Arg (I :1: 1)	7.8	~220
DPPC:OA:Arg (1: 1 :1)	9.0	~25.4

Thus, a desired size of the liposomal vesicles can be prepared by varying the arginine concentration or the pH of the aqueous buffer solution.

EXAMPLE #20

Liposome Stability

Sterile liposomes may be prepared from the heat sterilized pre-liposome gel. Alternatively, the liposome gel or the liposomes may be sterile filtered through an appropriate sterilizing filter.

Liposomes prepared from DPPC:OA:Arg (1:1:2) at pH 8.0 were heat sterilized and stored at room temperature for approximately one year without adding antimicrobial agents and anti-oxidants. No bacterial growth, discoloration and precipitation were observed. Negative stain electron microscopic examination of the one year old liposomes revealed that the liposomal vesicles are stable.

EXAMPLE #21

Encapsulated sucrose latency was measured using C^{14}-sucrose encapsulated with the DPPC:OA:Arg (1:1:1) liposome system in aqueous phosphate buffer solution at pH 7.8. The result was presented in Table IV.

Table IV

% Sucrose Latency

Days	% Latency
0	100
1	97.4
3	93.4
7	91.4

Thus, the present liposome system has an excellent latency for drug delivery.

EXAMPLE #22

Efficiency of Encapsulation

A number of drugs were encapsulated with 10 mg/ml DPPC:oleic acid:Arg (1:1:1) liposomes to illustrate medicament encapsulation for use as a drug delivery system. The results are presented in Table V.

Table V

Entrapment of Drugs

Drugs	pH	% Entrapment
Flurbiprofen	7.8 PBS	90%
Dipivalyl Epinephrine	7.1 PBS	80%

Example #23

Lyophilized Liposomes

Oleic acid, 30.0 gm, and 7.5 gm of cholesterol U.S.P. were confected. Then 75.0 gm of phosphatide type 20 powder (Asahi Chemical Co.) was mixed with the oleic acid/cholesterol mixture until an homogeneous paste was formed.

Then 15.0 gm of arginine (free base) was dissolved in 183 gm of distilled, deionized water. This arginine solution was mixed slowly with the lipid paste to form a homogeneous gel. The gel pH was adjusted to 7.4 using 5.0

N HCl.

A 10.0 gm aliquot of this pre-liposome gel was transferred to a 10 ml vial and lyophilized. The resulting powder formed liposomes when diluted with 5 ml of phosphate buffered saline.

EXAMPLE #24

Acid Stable Liposome Preparations

An example of acid stable liposomes employing this invention is illustrated by liposomes prepared with the following materials: distearoylphosphatidylcholine dipalmitoyl phosphatidylcholine: oleic acid, arginine, and cholesterol. These materials were combined in a molar ratio of 1:2:2:2:0.2 as follows: Cholesterol, 20 mg., was mixed with 144 mg. of oleic acid and treated to 40°C. DSPC, 200 mg., and 400 mg. of DPPC was added and mixed at 40°C. The mixture was stirred until a uniform homogeneous paste was formed.

Arginine, 88 mg., was dissolved in 1.15 g. of deionized distilled water. This arginine solution was added to the lipid paste and mixed at about 45°C until a homogeneous pre-liposome gel formed. The pH of the gel was adjusted to various pH levels with 0.1N HCl. The gel was diluted 10-fold with 0.9% NaCl, forming vesicles.

Liposome Stability at pH 4.4

Time/Days	Size/($NM \times 10^{3}$)
0	1.024
3	1.136
7	1.127

CLAIMS (BE, CH, DE, FR, GB, IT, LI, LU, NL and SE)

1. A liposome composition made by dispersing in an aqueous medium in a manner adequate to form liposomes:

a. liposome-forming material;

b. a hydrating agent wherein the hydrating agent is present in a molar ratio of between 1:20 and 1:0.05 relative to the liposome-forming material; and

c. optionally material to be encapsulated.

2. The composition of claim 1 wherein the hydrating agent is an alpha amino acid having an omega subsitution which is a carboxylate, amino, guanidino function or a pharmaceutically acceptable salt thereof or a compound of the formula

$$X\text{-}(CH_2)n\text{-}Y \quad I$$

wherein

X is $H_2N\text{-}C(NH)\text{-}NH\text{-}$, $H_2N\text{-}$, $ZO_3S\text{-}$, $Z_2O_3P\text{-}$, or $ZO_2C\text{-}$ wherein Z is H or an inorganic or organic cation;

Y is $-CH(NH_2)\text{-}CO_2H$, $-NH_2$, $-NH\text{-}C(NH)\text{-}NH_2$, $-COOH$, $CH(NH_2)SO_3Z$ or $ZH(NH_2)PO_3Z_2$ wherein Z is defined above; and

n is the integer 1-10; or

a pharmaceutically acceptable salt thereof.

3. The composition of claim 2 wherein the hydrating agent is present in an amount between 1:2 to 1:0.5 molar ratio relative to the liposome-forming material.

4. The composition of claim 3 wherein said hydrating agent is arginine, homoarginine, or their N-acyl derivatives, gamma-aminobutyric acid, asparagine, lysine, ornithine, glutamic acid, aspartic acid or a compound of the formulae:

$H_2NC(NH)-NH-(CH_2)_n-CH(NH_2)COOH$ II,

$H_2N-(CH_2)_n-CH(NH_2)COOH$ III,

$H_2N-(CH_2)_n-NH_2$ IV,

$H_2NC(NH)-NH-(CH_2)_n-NH-CH(NH)-NH_2$ V,

$HOOC-(CH_2)_n-CH(NH_2)COOH$ VI,

$HOOC-(CH_2)_n-COOH$ VII,

$HO_3S-(CH_2)_n-CH(NH_2)COOH$ VIII,

$H_2O_3S-(CH_2)_n-CH(NH_2)COOH$ IX,

$HO_3S-(CH_2)_n-CH(NH_2)SO_3H$ X, or

$H_2O_3S-(CH_2)_n-CH(NH_2)PO_3H_2$ XI

wherein n is 2-4, or a pharmaceutically acceptable salt thereof.

5. The product of claim 4 wherein said hydrating agent is arginine, homoarginine, gamma-aminobutyric acid, lysine, or ornithine or a pharmaceutically acceptable salt thereof.

6. The composition of claim 4 wherein said hydrating agent is glutamic acid or aspartic acid or a pharmaceutically acceptable salt thereof.

7. A composition capable of forming liposomes when dispersed in an aqueous medium and the liposomes made therefrom, which composition comprises:

a. liposome-forming material;

b. a hydrating agent wherein the hydrating agent is present in a molar ratio of between 1:20 and 1:0.05 relative to the liposome-forming material;

c. optionally material to be encapsulated; and

d. water in an amount up to 300 moles relative to the solids.

8. The composition of claim 7 wherein the hydrating agent is an alpha amino acid having an omega substitution which is a carboxylate, amino, or guanidino function or a pharmaceutically acceptable salt thereof, or a compound of the formula:

$$X\text{-}(CH_2)_n\text{-}Y \qquad I$$

wherein

X is $H_2N\text{-}C(NH)\text{-}NH\text{-}$, $H_2N\text{-}$, $ZO_3S\text{-}$, $Z_2O_3P\text{-}$, or $ZO_2C\text{-}$ wherein Z is H or an inorganic or organic cation;

Y is $\text{-}CH(NH_2)\text{-}CO_2H$, $\text{-}NH_2$, $\text{-}NH\text{-}C(NH)\text{-}NH_2$, -COOH, $CH(NH_2)SO_3Z$ or $ZH(NH_2)PO_3Z_2$ wherein Z is defined above; and

n is the integer 1-10; or

a pharmaceutically acceptable salt thereof.

9. The composition of claim 8 wherein the hydrating agent is present in an amount between 1:2 to 1:0.5 molar ratio relative to the liposome-forming material.

10. The product of claim 9 wherein said hydrating agent is arginine, homoarginine, or their N-acyl derivatives, gamma-aminobutyric acid, asparagine, lysine, ornithine, glutamic acid, aspartic acid or a compound of the formulae:

$$H_2NC(NH)\text{-}NH\text{-}(CH_2)_n\text{-}CH(NH_2)COOH \qquad II,$$

$$H_2N\text{-}(CH_2)_n\text{-}CH(NH_2)COOH \qquad III,$$

$$H_2N-(CH_2)_n-NH_2 \quad IV,$$

$$H_2NC(NH)-NH-(CH_2)_n-NH-CH(NH)-NH_2 \quad V,$$

$$HOOC-(CH_2)_n-CH(NH_2)COOH \quad VI,$$

$$HOOC-(CH_2)_n-COOH \quad VII,$$

$$HO_3S-(CH_2)_n-CH(NH_2)COOH \quad VIII,$$

$$H_2O_3S-(CH_2)_n-CH(NH_2)COOH \quad IX,$$

$$HO_3S-(CH_2)_n-CH(NH_2)SO_3H \quad X, \text{ or}$$

$$H_2O_3S-(CH_2)_n-CH(NH_2)PO_3H_2 \quad XI$$

wherein n is 2-4, or a pharmaceutically acceptable salt thereof.

11. The composition of claim 10 wherein said hydrating agent is arginine, homoarginine, gamma-aminobutyric acid, lysine, or ornithine or a pharmaceutically acceptable salt thereof.

12. The composition of claim 10 wherein said hydrating agent is glutamic acid or aspartic acid or a pharmaceutically acceptable salt thereof.

13. A means for forming stable liposomes wherein the means comprises adding to liposome-forming materials before or at the time they are dispersed in an aqueous medium, a hydrating agent in a molar ratio of 1:20 to 1:0.05 relative to the liposome-forming material.

14. A substantially dry composition capable of forming liposomes when dispersed in an aqueous medium wherein the composition comprises:

(a) a liposome forming material;

(b) a hydrating agent where the hydrating agent is present in a molar ratio between 1:20 and 1:0.05 relative to the liposome forming material; and

(c) optionally material to be encapsulated,

which composition is prepared by mixing in an aqueous medium the materials of (a), (b) and (c), and dehydrating said mixture to give said substantially dry composition.

15. The composition of claim 14 wherein the hydrating agent is an alpha amino acid having an omega substitution which is a carboxylate, amino, or guanidino function or a pharmaceutically acceptable salt thereof, or a compound of the formula:

$$X\text{-}(CH_2)_n\text{-}Y \qquad I$$

wherein

X is H_2N-C(NH)-NH-, H_2N-, ZO_3S-, Z_2O_3P-, or ZO_2C- wherein Z is H or an inorganic or organic cation;

Y is -CH(NH_2)-CO_2H, -NH_2, -NH-C(NH)-NH_2, -COOH, CH(NH_2)SO_3Z or ZH(NH_2)PO_3Z_2 wherein Z is defined above; and

n is the integer 1-10; or

a pharmaceutically acceptable salt thereof.

16. The composition of claim 15 wherein the hydrating agent is present in an amount between 1:2 to 1:0.5 molar ratio relative to the liposome-forming material.

17. The product of claim 16 wherein said hydrating agent is arginine, homoarginine, or their N-acyl derivatives, gamma-aminobutyric acid, asparagine, lysine, ornithine, glutamic acid, aspartic acid or a compound of the formulae:

$H_2NC(NH)-NH-(CH_2)_n-CH(NH_2)COOH$ II,

$H_2N-(CH_2)_n-CH(NH_2)COOH$ III,

$H_2N-(CH_2)_n-NH_2$ IV,

$H_2NC(NH)-NH-(CH_2)_n-NH-CH(NH)-NH_2$ V,

$HOOC-(CH_2)_n-CH(NH_2)COOH$ VI,

$HOOC-(CH_2)_n-COOH$ VII,

$HO_3S-(CH_2)_n-CH(NH_2)COOH$ VIII,

$H_2O_3S-(CH_2)_n-CH(NH_2)COOH$ IX,

$HO_3S-(CH_2)_n-CH(NH_2)SO_3H$ X, or

$H_2O_3S-(CH_2)_n-CH(NH_2)PO_3H_2$ XI

wherein n is 2-4, or a pharmaceutically acceptable salt thereof.

18. The composition of claim 17 wherein said hydrating agent is arginine, homoarginine, gamma-aminobutyric acid, lysine, or ornithine or a pharmaceutically acceptable salt thereof.

19. The composition of claim 17 wherein said hydrating agent is glutamic acid or aspartic acid or a pharmaceutically acceptable salt thereof.

CLAIMS (AT)

1. A method for making a liposome composition which method comprises dispersing in an aqueous solution in a manner adequate to form liposomes:

a. liposome-forming material;

b. a hydrating agent wherein the hydrating agent is present in a molar ratio of between 1:20 and 1:0.05 relative to the liposome-forming material; and

c. optionally material to be encapsulated.

2. The method of claim 1 wherein the hydrating agent is an alpha amino acid having an omega subsitution which is a carboxylate, amino, guanidino function or a pharmaceutically acceptable salt thereof or a compound of the formula

$$X\text{-}(CH_2)n\text{-}Y \quad I$$

wherein

X is $H_2N\text{-}C(NH)\text{-}NH\text{-}$, $H_2N\text{-}$, $ZO_3S\text{-}$, $Z_2O_3P\text{-}$, or $ZO_2C\text{-}$ wherein Z is H or an inorganic or organic cation;

Y is $-CH(NH_2)-CO_2H$, $-NH_2$, $-NH-C(NH)-NH_2$, $-COOH$, $CH(NH_2)SO_3Z$ or $ZH(NH_2)PO_3Z_2$ wherein Z is defined above; and

n is the integer 1-10; or

a pharmaceutically acceptable salt thereof.

3. The method of claim 2 wherein the hydrating agent is present in an amount between 1:2 to 1:0.5 molar ratio relative to the liposome-forming material.

4. The method of claim 3 wherein said hydrating agent is arginine, homoarginine, or their N-acyl derivatives, gamma-aminobutyric acid, asparagine, lysine, ornithine, glutamic acid, aspartic acid or a compound of the formulae:

$H_2NC(NH)-NH-(CH_2)_n-CH(NH_2)COOH$ II,

$H_2N-(CH_2)_n-CH(NH_2)COOH$ III,

$H_2N-(CH_2)_n-NH_2$ IV,

$H_2NC(NH)-NH-(CH_2)_n-NH-CH(NH)-NH_2$ V,

$HOOC-(CH_2)_n-CH(NH_2)COOH$ VI,

$HOOC-(CH_2)_n-COOH$ VII,

$HO_3S-(CH_2)_n-CH(NH_2)COOH$ VIII,

$H_2O_3S-(CH_2)_n-CH(NH_2)COOH$ IX,

$HO_3S-(CH_2)_n-CH(NH_2)SO_3H$ X, or

$H_2O_3S-(CH_2)_n-CH(NH_2)PO_3H_2$ XI

wherein n is 2-4, or a pharmaceutically acceptable salt thereof.

5. The method of claim 4 wherein said hydrating agent is arginine, homoarginine, gamma-aminobutyric acid, lysine, or ornithine or a pharmaceutically acceptable salt thereof.

6. The method of claim 5 wherein said hydrating agent is glutamic acid or aspartic acid or a pharmaceutically acceptable salt thereof.

7. A method for making a liposome composition which method comprises dispersing in an aqueous solution in a manner adequate to form liposomes, a composition comprised of:

a. liposome-forming material;

b. a hydrating agent wherein the hydrating agent is present in a molar ratio of between 1:20 and 1:0.05 relative to the liposome-forming material; and

c. water in an amount up to 300 moles relative to the solids; and

d. optionally, material to be encapsulated.

8. The method of claim 7 wherein the hydrating agent is an alpha amino acid having an omega subsitution which is a carboxylate, amino, or guanidino function or a pharmaceutically acceptable salt thereof or a compound of the formula

$$X\text{-}(CH_2)n\text{-}Y \quad I$$

wherein

X is $H_2N\text{-}C(NH)\text{-}NH\text{-}$, $H_2N\text{-}$, $ZO_3S\text{-}$, $Z_2O_3P\text{-}$, or $ZO_2C\text{-}$ wherein Z is H or an inorganic or organic cation;

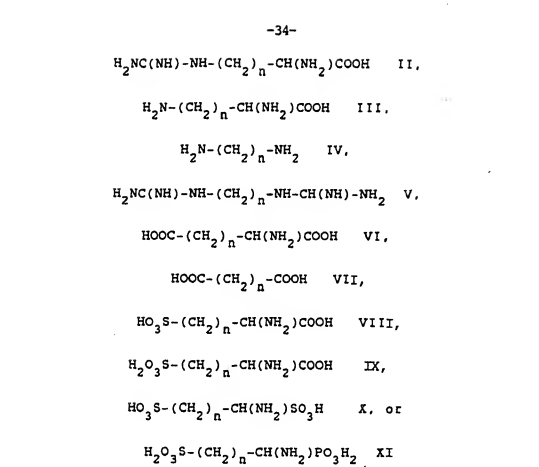
Y is $-CH(NH_2)\text{-}CO_2H$, $-NH_2$, $-NH\text{-}C(NH)\text{-}NH_2$, $-COOH$, $CH(NH_2)SO_3Z$ or $ZH(NH_2)PO_3Z_2$ wherein Z is defined above; and

n is the integer 1-10; or

a pharmaceutically acceptable salt thereof.

9. The method of claim 8 wherein the hydrating agent is present in an amount between 1:2 to 1:0.5 molar ratio relative to the liposome-forming material.

10. The method of claim 9 wherein said hydrating agent is arginine, homoarginine, or their N-acyl derivatives, gamma-aminobutyric acid, asparagine, lysine, ornithine, glutamic acid, aspartic acid or a compound of the formulae:



$H_2NC(NH)-NH-(CH_2)_n-CH(NH_2)COOH$ II,

$H_2N-(CH_2)_n-CH(NH_2)COOH$ III,

$H_2N-(CH_2)_n-NH_2$ IV,

$H_2NC(NH)-NH-(CH_2)_n-NH-CH(NH)-NH_2$ V,

$HOOC-(CH_2)_n-CH(NH_2)COOH$ VI,

$HOOC-(CH_2)_n-COOH$ VII,

$HO_3S-(CH_2)_n-CH(NH_2)COOH$ VIII,

$H_2O_3S-(CH_2)_n-CH(NH_2)COOH$ IX,

$HO_3S-(CH_2)_n-CH(NH_2)SO_3H$ X, or

$H_2O_3S-(CH_2)_n-CH(NH_2)PO_3H_2$ XI

wherein n is 2-4, or a pharmaceutically acceptable salt thereof.

11. The method of claim 10 wherein said hydrating agent is arginine, homoarginine, gamma-aminobutyric acid, lysine, or ornithine or a pharmaceutically acceptable salt thereof.

12. The method of claim 10 wherein said hydrating agent is glutamic acid or aspartic acid or a pharmaceutically acceptable salt thereof.

13. A method of preparing a substantially dry composition capable of forming liposomes when dispersed in an aqueous medium wherein the composition comprises:

(a) a liposome forming material;

(b) a hydrating agent where the hydrating agent is present in a molar ratio between 1:20 and 1:0.05 relative to the liposome forming material; and

(c) optionally material to be encapsulated,

which method comprises mixing in an aqueous medium the materials of (a), (b) and (c), and dehydrating said mixture to give said substantially dry composition.

14. The method of claim 13 wherein the hydrating agent is an alpha amino acid having an omega substitution which is a carboxylate, amino, or guanidino function or a pharmaceutically acceptable salt thereof, or a compound of the formula:

$$X\text{-}(CH_2)_n\text{-}Y \qquad I$$

wherein

X is $H_2N\text{-}C(NH)\text{-}NH\text{-}$, $H_2N\text{-}$, $ZO_3S\text{-}$, $Z_2O_3P\text{-}$, or $ZO_2C\text{-}$ wherein Z is H or an inorganic or organic cation;

Y is $-CH(NH_2)\text{-}CO_2H$, $-NH_2$, $-NH\text{-}C(NH)\text{-}NH_2$, $-COOH$, $CH(NH_2)SO_3Z$ or $ZH(NH_2)PO_3Z_2$ wherein Z is defined above; and

n is the integer 1-10; or

a pharmaceutically acceptable salt thereof.

15. The method of claim 14 wherein the hydrating agent is present in an amount between 1:2 to 1:0.5 molar ratio relative to the liposome-forming material.

16. The method of claim 15 wherein said hydrating agent is arginine, homoarginine, or their N-acyl derivatives, gamma-aminobutyric acid, asparagine, lysine, ornithine, glutamic acid, aspartic acid or a compound of the formulae:

$H_2NC(NH)-NH-(CH_2)_n-CH(NH_2)COOH$ II,

$H_2N-(CH_2)_n-CH(NH_2)COOH$ III,

$H_2N-(CH_2)_n-NH_2$ IV,

$H_2NC(NH)-NH-(CH_2)_n-NH-CH(NH)-NH_2$ V,

$HOOC-(CH_2)_n-CH(NH_2)COOH$ VI,

$HOOC-(CH_2)_n-COOH$ VII,

$HO_3S-(CH_2)_n-CH(NH_2)COOH$ VIII,

$H_2O_3S-(CH_2)_n-CH(NH_2)COOH$ IX,

$HO_3S-(CH_2)_n-CH(NH_2)SO_3H$ X, or

$H_2O_3S-(CH_2)_n-CH(NH_2)PO_3H_2$ XI

wherein n is 2-4, or a pharmaceutically acceptable salt thereof.

17. The method of claim 16 wherein said hydrating agent is arginine, homoarginine, gamma-aminobutyric acid, lysine, or ornithine or a pharmaceutically acceptable salt thereof.

18. The method of claim 16 wherein said hydrating agent is glutamic acid or aspartic acid or a pharmaceutically acceptable salt thereof.

European Patent Office

EUROPEAN SEARCH REPORT

0211647

Application number

DOCUMENTS CONSIDERED TO BE RELEVANT

EP 86306014.1

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl 4)
A	DE - A1 - 2 706 705 (TAKEDA CHEMICAL INDUSTRIES) * Claims 1,17-19,26; page 11, lines 16-17; page 12, lines 29-31; examples 39,40 * --	1-5,7-11,13-18	A 61 K 9/50 A 61 K 9/52 A 61 K 9/10 A 61 K 47/00 B 01 J 13/02
A	EP - A1 - 0 120 722 (PARFUMS CHRISTIAN DIOR) * Examples 7,8,9 * --	1-4,7-10,13	/A 61 K 31/195
X	EP - A1 - 0 087 993 (PARFUMS CHRISTIAN DIOR) * Claims 1,7,8; examples 11,12; page 17, lines 4-13 * --	1-4,6-10,12,13	
X	EP - A2 - 0 092 453 (THE LIPOSOME CORPORATION) * Abstract; claims 1,10,14,16, 19,28,29,31,32; page 11, line 23 - page 14, line 18; page 35, line 32 - page 36, line 12; sections 6.1-6.3 * --	1,7,13	TECHNICAL FIELDS SEARCHED (Int. Cl 4) A 61 K 9/00 B 01 J 13/00
X	US - A - 3 957 971 (W.S.OLEUIACZ) * Abstract; claims 1,11,12; column 5, lines 18-45 * --	1-4,6-13	
X	US - A - 3 932 657 (Y.E.RAHMANN) * Abstract; column 3, line 44 - column 4, line 33 * --	1,7,13	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
VIENNA	14-11-1986	MAZZUCCO

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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EUROPEAN SEARCH REPORT

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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int Cl.4)
X	WO - A1 - 84/02 076 (FLUID-CARBON INTERNATIONAL AB) * Abstract; claims 1,2,4,19,26-28,32; examples 4,6 *	1,7,13	
X	GB - A - 2 047 535 (A.NATTERMANN & CIE GMBH) * Claims 1,3,5; examples 1,2 *	1,7,13, 14	
X	GB - A - 2 041 871 (FARMITALIA CARLO ERBA S.P.A.) * Abstract; claims 7,8; examples 1,2 *	1,7,13, 14	
X	DE - A1 - 2 856 333 (A.NATTERMANN & CIE GMBH) * Claims 1-4,7-9; examples 2,4 *	1-5,7-11,13-18	TECHNICAL FIELDS SEARCHED (Int. Cl.4)
A	AT - B - 356 278 (F.HOFFMANN - LA ROCHE & CO AKTIENGESELLSCHAFT) * Claim 1 *	1,2,7, 8,13	
A	DE - A1 - 2 656 333 (K.THEURER) * Example 2 *	1,2,4, 5,7,8, 10,11, 13	
A	CH - A - 498 627 (K.LARSSON) * Claims 1,2; column 3, lines 22-31,60-67; column 4, lines 22-26 *	1,7,13	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
VIENNA	14-11-1986	MAZZUCCO

CATEGORY OF CITED DOCUMENTS

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T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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European Patent Office

EUROPEAN SEARCH REPORT

Application number

EP 86306014.1

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	US - A - 4 174 296 (G.S.KASS) * Claim 1 * --	1	
A	EP - A2 - 0 130 577 (DAIICHI SEIYOKU CO. LTD.) * Pages 4,5,8; abstract; claims 1,4-7,11-13 * --	1,7,13	
A	EP - A2 - 0 102 324 (CIBA-GEIGY AG) * Abstract; claims 1,17; page 23, lines 18-23; page 24, line 22 - page 25, line 15 * ----	1,2,7, 8,13, 14	
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
VIENNA	14-11-1986	MAZZUCCO

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
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(54) **Hautwirksame Pharmaka mit Liposomen als Wirkstoffträger.**

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Beschreibung

Die Erfindung betrifft Medikamente zur Therapie von Erkrankungen der Haut, die gegebenenfalls schon an sich für die Behandlung von Hautkrankheiten bekannte Wirkstoffe enthalten, und die als Externa im Bereich der erkrankten Hautstelle angewendet werden.

Zur Lokaltherapie von Hautkrankheiten werden in der Regel Salben, Cremes, Lotionen oder Tinkturen verwendet, in die bestimmte Wirkstoffe eingearbeitet sind. Für den therapeutischen Effekt dieser Wirkstoffe ist Voraussetzung, daß sie aus der entsprechenden Grundlage in ausreichender Menge freigegeben werden. Nur wenn dies der Fall ist, können die Wirkstoffe dann in die Haut eindringen und dorthin gelangen, wo ihre Wirkungsqualitäten benötigt werden.

Neuartige Arzneistoffträger sind die Liposomen. Als solche werden kugelförmige Gebilde aus einer oder mehreren Lipiddoppelschichten mit wässrigen Innenraum bezeichnet, und die sich aus Phospholipiden, z. B. Lecithin, unter anderem durch deren mechanische Feinverteilung herstellen lassen. Herstellungsweisen von Liposomen und deren Verwendung als Arzneimittel- oder Kosmetikmittelträger, wobei die Wirkstoffe in den Innenräumen der Liposomen eingelagert sind, sind in US-A 3 957 971, in DE-A 28 18 655, in DE-A 28 34 308 oder in GB-A-2 013 609 angegeben. Aus "Pharmazie in unserer Zeit" 11, (1982) 97 bis 108, ist praktisch der derzeitige Stand des Wissens bezüglich Herstellung von Liposomen und deren Anwendung als Arzneimittelträger ersichtlich. Danach sind Applikationsarten wie intravenöse, intramuskuläre und subcutane Injektionen und auch orale Verabreichungen von arzneimittelhaltigen Liposomen getestet worden. Prüfungen von medikamentösen Formulierungen mit Liposomen als Träger zu lokalen Anwendungen auf der Haut, z. B. bei Hautkrankheiten, standen möglicherweise Vorurteile infolge der Kenntnisse des Hautaufbaues und die vielfältigen Erfahrungen mit Applikationen auf der Haut entgegen, da in der Epidermis unter dem Stratum corneum, der die Oberfläche der Haut bildenden Hornschicht, eine dichter gebaute Zellage, das Stratum conjunctum oder compactum, liegt, deren Funktion die Erschwerung bzw. Verhinderung der Penetration von Stoffen von außen ist.

Auch Pharmazie, Bd. 39, 9/1984, 627-629, beschreibt "Liposomen als Wirkstoffträger in der percutanen Therapie", für Triamcinolon als Modellsubstanz mit drei- bis viermal höherer Resorption percutan.

Die am häufigsten verwendeten Wirkstoffe zur äußeren Behandlung von Hautkrankheiten sind Kortikosteroide und Trihydroxianthracene. So beschreibt die Rote Liste 1983 unter Nr. 31 370 Psoradexan®-Creme (Dithranol/Harnstoff) zur Anwendung bei subakuter und chronischer Psoriasis. Wegen ihres geringen Penetrationsvermögens durch die Haut nach Liberation aus den Trägergrundlagen, müssen diese Stoffe, um einen ausreichenden therapeutischen Effekt zu erzielen in hoher Konzentration und in hohem Überschuß in z. B. Salben oder Cremes vorliegen.

Aufgabe und Gegenstand der Erfindung

Es war daher Aufgabe der Erfindung hautwirksame Pharmaka zu finden, mit denen die Wirkstoffe schneller in die Haut dringen bzw. diese durchdringen können, um so Medikamente zur Verfügung zu haben, die die Wirkungssubstanzen ständig und in ausreichender Menge an den Wirkort geben.

Hautwirksame Stoffe, wie z. B. Kortikosteroide oder Anthracentriole, lassen sich relativ einfach mit Liposomen kombinieren, d. h. in diese einschließen. Überraschenderweise wurde gefunden, daß mit so beladenen Liposomen, in einer streichfähigen Form z. B. als Salben oder Cremes auf die Haut gebracht, die Penetration der Wirkstoffe wesentlich schneller erfolgt als im Vergleich aus einer üblichen Wirkstoff-Salben-Formulierung.

Wie weiter gefunden wurde, läßt sich Harnstoff, von dem zur Therapie von Hauterkrankungen selbst eine ganze Reihe von Wirkungsqualitäten, z. B. als Keratolytikum, bekannt sind, vorteilhaft mit in die Liposomenpräparate ein arbeiten, wobei Mischungen von Harnstoff und Hydroxyanthracenen (Anthratriole) und gegebenfalls weiteren hautwirksamen Stoffen, wie z. B. Steroiden mit den Liposomen bzw. den Phospholipidgemischen als deren Vorstadium zu hautwirksamen Medikamenten formuliert werden.

Beispielsweise werden mit erfindungsgemäßen Harnstoff-Dithranol-Zubereitungen etwa 3 bis 4 mal höhere Wirkstoff-Haut Penetrationen als mit bisher üblichen Formulierungen gemessen. Somit ermöglichen die neuen Formulierungen erhebliche Einsparungen an Wirkstoffmengen. Durch die Mengeneinsparung werden auch andere Probleme, wie z. B. Verschmutzung von Textilien, die insbesondere bei Anwendung farbiger Substanzen oder farbiger Metabolite, wie z. B. Oxidationsprodukten der Anthratriole, auftreten, entschärft bzw. gelöst.

Durchführung der Erfindung

Die Herstellung der erfindungsgemäß anzuwendenden Liposomenzubereitung aus den Aufbaukomponenten der Liposomen, den Phospholipiden, insbesondere den Phosphatidylcholinen, wie den Lecithinen, einer in der Natur vorkommenden Gruppe von Phospholipiden, beispielsweise Lecithin aus Sojabohnen, dem gebräuchlichsten Rohstoff zur Herstellung von Lecithin, sowie weiteren Zusatzstoffen, wie Sterinen, z. B. dem Cholsterin, und den hautwirksamen Stoffen, dem Harnstoff und den Hydroxyanthracenen, kann nach verschiedenen Methoden, wie sie beispielsweise in den oben angegebenen Literaturstellen beschrieben sind, vorgenommen werden. Das für die Herstellung der Liposomenträger anzuwendende Verhältnis der in der Lipidausgangsmischung vorhandenen Substanzen, von z. B. Lecithin zu z. B. Cholesterin, kann in den Bereichen 10 : 0,1 bis etwa 1 : 1, vorwiedend bei 10 : 1 bis 1 : 1, insbesondere bei 5 : 1 bis 2 : 1 liegen. Auch weitere Substanzen, z. B. Dialkylphosphate oder Sphingomyelin oder Hilfsstoffe wie Zucker- bzw. Aminozuckerderivate, z. B. zur Stabilisierung der Liposomenmembran, und Antioxidantien können bei der Präparierung mitverwendet werden.

Die Herstellung kann beispielsweise nach der bekannten Film-Dispersions-Methode, einer schonenden Eindampfung, z. B. im Rotationsverdampfer, der Lipid-Wirkstoff-Mischung in Chloroform-Alkohol-Lösung vorgenommen werden, welcher zur Herstellung besonders kleiner Partikel mit relativ enger Größenverteilung eine Ultraschallbehandlung nachgeschaltet werden kann. Durch Zentrifugieren und Waschen können die wirkstoffhaltigen Liposomen dann isoliert und gereinigt werden. Die Partikelgröße der nach solchen Verfahren erhaltenen Liposomen kann stark variieren und im Bereich von einem oder mehreren Micrometern bis hinunter zu etwa 10 nm liegen. Für die Verwendung als hautwirksame Pharmakaträger haben die Liposomen vorzugsweise Durchmesser von 20 bis 50 nm.

Wirkungssubstanzen, die mit den obengenannten Aufbaukomponenten der Liposomen erfindungsgemäße hautwirksame Pharmaka bilden, gehören z. B. der großen Gruppen der Antibiotika oder der Sulfonamide an, oder sind insbesondere solche aus der Gruppe der Kortikosteroide und Hydroxyanthracene. Die therapeutischen Wirkungen dieser Stoffe sind bekanntermaßen insbesondere bei der Behandlung entzündlicher, ekzematöser oder allergischer Hauterkrankungen angezeigt. Durch Kombination von Wirkstoffen aus den verschiedenen Substanzgruppen wird noch eine therapeutische Wirkungssteigerung erzielt. Die Verwendung von Harnstoff bei der Herstellung wirkstoffhaltiger Liposomen in Kombination mit Hydroxyanthracenen und gegebenfalls weiter mit Kortikosteroiden, führt zu therapeutisch wertvollen Formulierungen. Die Vorteile der erfindungsgemäßen Medikamente gegenüber bekannten Medikamenten mit gleichen Wirksubstanzen, sind durch die verbesserte Penetration durch die Haut, damit einer höheren Verfügbarkeit des Arzneistoffes am Wirkort und somit einer deutlichen Senkung der anzuwendenden Wirkstoffmenge gegeben, was sich in einer Verringerung von Nebenwirkungen und wesentlichen Verbilligung des Medikaments niederschlägt.

Erfindungsgemäß einsetzbare Kortikosteroide sind beispielsweise Fludrocortison, Fluocortolon, Fluorandrenolon, Triamcinolon, Methylprednisolon; Anthracentriole, die in den erfindungsgemäßen Formulierungen eingesetzt werden, und die als Dermatica oder Antiseptika bekannt sind, sind die Hydroxyanthracene 1,8,9-Anthracentriol, auch als 1,8,9-Anthratriol bzw. 1,8-Dihydroxianthranol-(9) und unter dem von der WHO vorgeschlagenen Freinamen Dithranol bekannt, oder 1,2,10-Anthracentriol, das auch als 1,2,10-Anthratriol bekannt ist.

Auch für die transdermale Applikation von Wirkstoffen verschiedener Art bei Erkrankungen anderer Organismusteile als der Haut bzw. hautnaher Bereiche, ist die Verwendung von Medikamenten mit pharmakahaltigen Liposomen wegen deren besseren Hautpenetration und damit schnellerem Transport der Pharmaka deutlich wirkungsvoller.

Die Zubereitungen können auch noch Wirkstoffe für die beispielsweise eine Hautpenetration nicht angezeigt oder deren Penetrationsgeschwindigkeit nicht so kritisch ist, in bisher üblicher Zugabe enthalten.

Die zur äußeren Anwendung an der Haut hergestellten Zubereitungen sind disperse Systeme, die die Wirkstoff-Liposomen-Präparationen und gegebenenfalls weitere Wirkstoffe, gelöst, emulgiert oder suspendiert enthalten. Die Applikationsart der neuartigen hautwirksamen Medikamente mit pharmakahaltigen Liposomen wird im wesentlichen nach bisher bekannter Vorgehensweisen, d. h. beispielsweise als Salben oder Cremes durchgeführt.

Experimenteller Teil

1. Herstellung von Liposomen-Wirkstoff-Kombinationen

Chloroform/Äthanol 1 : 1 - oder Chloroform/Methanol 1 : 1 - Lösungen von Lecithin, Cholesterin und Wirkstoff, werden bei etwa 30 °C im Rotationsverdampfer vom Lösungsmittel unter Bildung eines dünnen Lipid-Wirkstoff-Films befreit. Anschließend wird der Film mit 8 molarer Calciumchloridlösung von 60 °C

versetzt und durch manuelles Schütteln die Ablösung der Liposomen von der Wandung erwirkt. Die so erhaltene Dispersion wird dann im Ultraschalldesintegrator weiter zerkleinert, anschließend zentrifugiert und dann die Liposomen-Wirkstoff-Kombination dreimal mit Calciumchloridlösung gewaschen.

Aus der folgenden Zusammenstellung sind die Zusammensetzungen hergestellter Liposomen in Gewichtsteilen Lecithin und Cholesterin und die von den eingesetzten Wirkstoffen Triamcinolon oder Dithranol eingebauten Anteile ersichtlich.

Lecithin (Gewichtsteile)		30	30	32.5	32.5	35	35
Cholesterin (Gewichtsteile)		13	10	13	10	10	7
Einbau von eingesetztem	Triamcinolon (in %)	41	32.4	56.2	42.8	62.1	49.8
	oder Dithranol (in %)	69.7	42.1	85.6	76.1	87.5	62.1

Patentansprüche

1. Hautwirksame, pharmazeutische Zubereitung zur äußeren Anwendung auf der Haut, die im wesentlichen aus Harnstoff und mindestens einem Wirkstoff aus der Gruppe der Hydroxyanthracene besteht, dadurch gekennzeichnet,
daß die Harnstoff-Hydroxyanthracen-Mischung in Liposomen eingearbeitet bzw. eingeschlossen ist.

2. Hautwirksame, pharmazeutische Zubereitung nach Anspruch 1, dadurch gekennzeichnet, daß das Hydroxyanthracen Dithranol ist.

3. Hautwirksame, pharmazeutische Zubereitung nach den Ansprüchen 1 und 2, dadurch gekennzeichnet, daß die Zubereitung neben der Harnstoff-Hydroxyanthracen-Mischung noch weitere bekannte hautwirksame Wirkstoffe in der Liposomen-Einarbeitung enthält.

4. Hautwirksame, pharmazeutische Zubereitung nach den Ansprüchen 1 bis 3, dadurch gekennzeichnet, daß der Zubereitung mit den in Liposomen eingeschlossenen Wirkstoffen auch Wirkstoffe in bisher üblicher Zubereitung beigegeben sind.

Claims

1. A dermatologically effective, pharmaceutical preparation for external use on the skin consisting largely of urea and at least one active substance from the group of hydroxyanthracenes, characterised in that the urea-hydroxyanthracene mixture is incorporated or enclosed in the liposomes.

2. A dermatologically effective, pharmaceutical preparation according to claim 1, characterised in that the hydroxyanthracene is dithranol.

3. A dermatologically effective, pharmaceutical preparation according to claims 1 and 2, characterised in that the preparation also has, apart from the urea-hydroxyanthracene mixture, further known dermatologically effective active substances which are incorporated in the liposomes.

4. A dermatologically effective, pharmaceutical preparation according to claims 1 to 3, characterised in that active substances as used in a hitherto conventional preparation are also added to the preparation containing active substances enclosed in the liposomes.

Revendications

1. Préparation pharmaceutique à action cutanée pour usage externe sur la peau, se composant essentiel-

lement d'urée et d'au moins une substance active du groupe des hydroxyanthracènes, caractérisée en ce que le mélange urée/hydroxyanthracène est incorporé ou inclus dans des liposomes.

2. Préparation pharmaceutique à action cutanée selon la revendication 1, caractérisée en ce que l'hydroxyanthracène est le dithranol.

3. Préparation pharmaceutique à action cutanée selon la revendication 1 ou 2, caractérisée en ce qu'elle contient, outre le mélange urée/hydroxyanthracène, d'autres substances actives connues à action cutanée, incorporées dans les liposomes.

4. Préparation pharmaceutique à action cutanée selon l'une quelconque des revendications **1** à 4, caractérisée en ce qu'il est adjoint, à la préparation contenant les substances actives incluses dans des liposomes, des substances actives en préparation jusqu'ici classique.

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A request for correction.(part of Claim nr 1 is missing). has been filed pursuant to Rule 88 EPC. A decision on the request will be taken during the proceedings before the Examining Division (Guidelines for Examination in the EPO, A-V, 3.).

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(54) Non-occulusive adhesive patch for applying medication to the skin.

(57) A non-occlusive medication patch (32) to be applied to the skin includes a porous self-supporting backing layer (10) to give the patch the required integrity and strength by acting as a supporting framework for other components, and a flexible hydrophilic pressure-sensitive adhesive reservoir (20) comprising a natural or synthetic polymer for the sustained release of medication to be absorbed topically through the skin into the body of a patient. The reservoir has two portions: first, an external coating layer (24) with an exposed lower skin-contacting surface (24b) that forms a pressure-sensitive bond with the skin, and second, an upper internal portion (24a) which infiltrates the porous backing (10) and becomes solidified therein after being applied so that the reservoir and the backing are unified, enabling the backing itself to act as a storage location for the medication-containing reservoir (20). The medication within the reservoir migrates over time from within the backing (10) through the lower coating layer (24b) and passes through the skin to provide sustained release of the medication into the body of a patient.

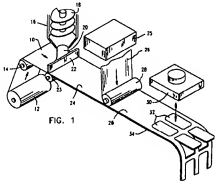

FIG. 1

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FIELD OF THE INVENTION

This invention relates to a non-occlusive adhesive patch for applying medication to the skin.

SUMMARY OF THE INVENTION

A non-occlusive medication-containing adhesive patch is provided for being applied to the skin for releasing a medication into the body of a patient. The patch has a porous backing layer that can be formed from a fibrous material, *e.g.,* non-woven fabric. To the backing is applied a flexible pressure-sensitive adhesive, typically a hydrocolloidal gel which serves as a reservoir for the sustained release of a medication which is uniformly distributed throughout the adhesive layer. The pressure-sensitive reservoir has two portions including, an external coating layer portion with an exposed surface for bonding to the skin and an internal portion that is infiltrated within the porous backing layer. The adhesive reservoir comprises a natural or synthetic polymer and a biomedically active medication dispersed therein. The internal portion of the hydrocolloidal reservoir which infiltrates the backing is solidified within the pores and interstices within the backing so that the reservoir and the backing are unified, enabling the backing itself to act as a storage location for the medication-containing reservoir. The gel reservoir is applied as a fluid. Solidification of the gel reservoir is delayed until after the gel has been applied to the porous backing layer.

THE FIGURES

Figure 1 is a perspective diagrammatic view illustrating a preferred method of forming products in accordance with the invention;

Figure 2 is a perspective view of the improved medication patch applied to the body;

Figure 3 is a plan view showing the medication patch packaged within in a pouch used as a shipping package;

Figure 4 is a cross-sectional view of the medication patch taken on line 4-4 of Fig. 3 with a portion of the liner sheet partially removed; and

Figure 5 is a greatly enlarged microscopic view of the medication patch and liner sheet taken on line 5-5 of Fig. 4.

DETAILED DESCRIPTION OF THE INVENTION

Refer now to Fig. 1 which illustrates diagrammatically the production of medication-applying patches in accordance with the invention. The backing sheet 10 is unwound continuously from a supply roll 12, passes upwardly in the figure over an idler roll 14 and then travels horizontally beneath a continuous processing mixer 16 where freshly prepared fluid hydrogel material at 20 is applied to the upper surface of the backing sheet 10.

The backing 10 is a porous self-supporting sheet of water insoluble polymeric material that provides strength and integrity for the adhesive patch as well as acting as a substrate for receiving and retaining a portion of the liquid hydrogel as will be described below.

One preferred backing sheet 10 is a lightweight, pliable strip composed, for example, from a nonwoven fabric which consists of polymeric fibers such as polyester, cotton or cellulose fibers bonded together with a sizing resin. The backing sheet 10 should be nonirritating to human skin. If desired, the backing sheet 10 can be coated on its back surface with a release coating such as a silicone release coating as described in patent 4,696,854 which is incorporated herein by reference. One suitable release coating is a 100% solids electron beam curable silicone such as Tego® Resin Acrylates/RC-Series RC 705 and RC 726 by Goldschmidt Chemical Corporation of Hopewell, Virginia. The preferred backing sheet 10 is a porous polymeric water insoluble nonwoven fibrous fabric. A suitable sizing material for bonding the fibers together is a latex resin.

The backing sheet 10 can comprise other stable, water insoluble flexible sheet materials. One preferred backing comprises a 0.14 mm strip of nonwoven fabric formed from a mixture of cellulose fibers derived from wood pulp and polyester fibers. The fibers are assembled loosely into the backing to maintain porosity. A unifying or sizing resin is applied to hold the fibers together. The sizing resin can comprise a nonirritating resin applied as a latex emulsion. One example is Hycar® 26477, a resin produced by B.F. Goodrich Co. of Brecksville, Ohio. Another suitable backing sheet is a nonwoven fabric comprising a wetlay cellulose and polyester nonwoven fabric containing as a sizing an acrylic latex emulsion resin, *e.g.,* product number N7601 by Dexter Corporation of Windsor Locks, Connecticut.

In another embodiment of the invention, the backing sheet 10 comprises a porous woven 0.12 mm acetate polymer cloth sometimes known as "silk cloth." Another form of backing sheet 10 is an open-cell plastic foam strip of low density polyethylene or polyvinyl acetate resin. Other backing sheets that can be used include woven cotton cloth or other cloth formed from a synthetic polymer. Suitable synthetic cloths include nylon, polyester, polyacetate. When the backing sheet 10 is a woven cloth, no sizing resin is needed. The backing sheet 10 is pervious to air so that the patch is non-occlusive to the skin.

The porosity of the backing sheet 10 is important because it provides openings for receiving the hydrocolloidal medication-containing reservoir and it helps to assure that the patch is non-occlusive to the skin. The infusion of the pressure-sensitive hydrocolloidal medication-containing reservoir into the backing sheet 10 is accomplished by controlling manufacturing parameters so as to keep the hydrocolloid sufficiently fluid to prenetrate the backing sheet 10 in spite of its tendency to thicken rapidly when applied. In order to prevent the consistency of the hydrogel from building too fast, *i.e.,* becoming too viscous to properly penetrate the backing sheet 10, a continuous processing mixer 16 (Fig. 1) which includes rotating auger 18 is chilled to help remove heat produced during mixing and keep the hydrogel cool until applied to the backing 10. This can be accomplished by providing the processing mixer 16 with a cooling jacket through which a coolant such as a chilled mixture of water and ethylene glycol is passed during operation. The components of the hydrogel are continuously added to the mixer 16 during operation. While any suitable mixer 16 can be used, one suitable mixer is a 12.7 cm continuous processing mixer manufactured by Teledyne Readco Company of York, Pennsylvania. The coolant passed through the processing mixer 16 can be maintained at about 0°C. The temperature of the fluid hydrogel 20 as it flows onto the exposed surface of the backing sheet 10 is important for controlling the infiltration of the coating into the backing sheet 10. The coolant will, under typical operating conditions, keep the extruded hydrogel 20 at a temperature of about 9°C to 14°C as it comes into contact with the backing 10. If deeper penetration is desired, the temperature of the hydrogel is lowered to about 9°C for a typical hydrogel formulation. If less penetration is wanted, the temperature is raised closer to 15°C.

The hydrogel produced by the processing mixer 16, which is in a chilled fluid condition, is expelled at 20 onto the exposed upper surface of the backing sheet 10 adjacent to a knife blade 22 of a knife coater which is held in spaced relationship above a rotatable support roll 23. The distance between the knife 22 and the roll 23 is controlled in any suitable manner, as by means of adjustment screws (not shown) or, if desired, the desired gap or spacing between the knife 22 and roll 23 can be preset to accommodate the backing sheet 10 and the thickness of the hydrogel coating 24 that is being applied to the exposed surface of the backing sheet 10.

In accordance with the invention, the medication-containing hydrogel 20 is applied so as to penetrate a substantial portion of the backing sheet 10, *e.g.,* typically between one-fourth to nine-tenths the thickness of the backing sheet 10. The penetration of the coating 24 into the backing 10 can be seen in Fig. 5. In this case the hydrogel coating 24 has penetrated about three-fourths of the way through the backing sheet 10 to provide an upper, *i.e.,* internal layer 24a of hydrocolloidal material within the pores between the fibers making up the porous backing sheet 10. The hydrogel material thus includes two layers as seen in Fig. 5; the external coating layer 24 with an exposed pressure-sensitive surface 24b and the upper internal portion 24a which infiltrates and becomes solidified within the backing in the interstices between the fibers that make up the porous backing sheet 10.

In one product with very good characteristics the backing sheet 10 is 0.14 mm in thickness and the external part of the coating layer 24 is 0.2 mm in thickness to provide a combined thickness for the patch when applied to the body of 0.34 mm. The external hydrogel layer 24 is purposely maintained relatively thin. The hydrocolloidal adhesive reservoir infiltrates into the backing to a depth of about 0.05 mm to 0.13 mm to provide a total hydrocolloid layer, including both the internal and external portions, of about 0.25 mm to 0.33 mm. Because of its thickness, the medication-containing reservoir provides a very adequate supply of medication to assure sustained release of the medication over an extended period of time, *e.g.,* six to eight hours or more. During use, the medication in the internal reservoir portion 24a stored within the backing sheet 10 migrates from within the backing sheet 10 through the external coating layer 24 and then passes through the skin to provide sustained release of the medication into the body of the patient.

After the hydrogel layer 24 is applied to the backing 10, the backing sheet continues moving toward the right as seen in Fig. 1 into close proximity with an oven or heater, in this case a radiant electric heater 25 which radiates heat onto the hydrogel coating layer 24, raising its temperature to about 60°C and causing it to cure, *i.e.,* to set up as a solid that is sufficiently stable to maintain its own shape and resist flow during storage or use. Once the heater 25 has warmed the hydrogel coating 24, it will be solidified and dimensionally stable. If curing is conducted without the application of heat, *e.g.,* at room temperature, it will take longer than when heat is used. A liner sheet 26 such as polyethylene coated paper is then applied

continuously by pressing it onto the exposed surface of the hydrogel layer 24 as the liner sheet 26 passes beneath a rotating roll 28. The assembled laminate 34 then moves further toward the right in the figure where a die press 30 stamps separate patches 32 from the sheet material.

The hydrogel 20, 24 comprises a hydrocolloidal dispersion of a hydrophilic natural or synthetic gel-forming polymer, a hydrophilic humectant, a biomedically active substance or medication, *i.e.,* a medicament, and a hydrophilic adhesive substance such as an aqueous dispersion of an acrylic adhesive.

The polymer can comprise a natural gum such as gum karaya, gum acacia, locust bean gum, guar gum, or other polysaccharide as well as synthetically formulated polysaccharides, *e.g.,* modified guar gum, maltodextrin, or celluloses such as carboxymethyl cellulose and carboxypropyl cellulose. The polymer can also comprise a synthetic polymer such as polyacrylamide and its cogeners or polyacrylic acid. Polyacrylamide is sold under the trademark Polytec 31x by Tecna Corp., Belleville, New Jersey.

The humectant can comprise a polyhydric alcohol such as glycerol, propylene glycol, ethylene glycol, or sorbitol.

The adhesive can comprise any suitable biocompatible hydrophilic adhesive such as a resin emulsion adhesive, *e.g.,* an acrylate emulsion adhesive or a copolymer of vinyl acetate and dioctyl maleate. The most outstanding results have been achieved with an acrylic emulsion adhesive. Other hydrophilic adhesives that can be used include an acrylic ester copolymer and a vinyl acetate resin.

Any of a variety of topical medications can be used in accordance with the present invention. The medications can be selected from a topical analgesic, anti-pruritic agent, anti-inflammatory agent, anesthetic agent, keratolytic agent and rubrefacient agent. When the patch is used as an analgesic, the analgesic can include trolamine salicylate, methyl salicylate, menthol, camphor, eucalyptus oil, spearmint oil, or a combination thereof. In other applications, the medication can include anti-pruritic agents or anti-inflammatory agent such as hydrocortisone, or anesthetic agents such as benzocaine or lidocaine. Also included are non-steroidal anti-inflammatory agents such as ibuprofen, especially the S-isomer of ibuprofen. Other medications that can be used include keratolytic agents such as salicylic acid, and rubrefacient agents such as capsicum.

In Fig. 2 the finished patch 32 is seen applied to the surface of the body with the backing 10 exposed and the pressure-sensitive hydrogel layer 24 bonded to the skin.

In Fig. 3 is shown a package containing the finished patch 32 as it appears during shipment and storage. The package 36 comprises a pouch including lower and upper layers of paper 35, 37 or other suitable packaging material such as metal foil coated paper which is sealed to itself along its edges, *e.g.,* at 36a, 36b to provide a sealed pouch containing the finished patch 32.

As shown in Figs. 4 and 5, the finished patch 32 includes the porous backing 10, the hydrogel coating including the lower, *i.e.,* external hydrogel coating layer 24 and the upper or internal portion 24a that permeates the backing 10. The upper surface 26a of the liner sheet 26 is a release surface for facilitating its removal. Before use, the liner sheet 26 is removed by pulling it off the patch as shown at the right in Fig. 4 to expose the pressure-sensitive surface of the layer 24 which is then applied to the skin as shown in Fig. 2.

During use, the upper or internal reservoir portion 24a that infiltrates the backing 10 and is solidified therein serves to store the medication within the backing 10 so that the medication migrates over time from its location at 24a within the backing 10 through the external coating layer 24 and then passes through the skin to provide sustained release of the medication into the body of the patient.

The porosity of the backing 10 combined with the water compatibility of the hydrocolloidal dispersion also makes the patch non-occlusive so that moisture from the body can evaporate through the patch into the atmosphere. The moisture vapor transmission rate (MVTR) of the skin alone under various conditions is typically from about 70 to about 149 $g/m^2/24hr$ while the medication applying patch of the present invention is about 612 to 1952 $g/m^2/24hr$. This shows that the invention is non-occlusive because in a given period of time about 8 to 14 times more moisture vapor is transmitted through the patch of the present invention than through the skin. Prior medication-applying patches that employed a rubber backing allow virtually no moisture evaporation from the skin. By contrast, the non-occlusive patch of the present invention will not interfere with moisture evaporation from the skin. This is important because the evaporation of moisture from the skin helps the skin to act in its normal capacity as a barrier to externally applied compounds which, if absorbed in excessive amounts, can produce toxic reactions or skin irritation. The invention thus enables the barrier function of the *stratum corneum* to be maintained.

When used as an analgesic patch, the present invention provides outstanding results in relieving pain such as arthritis pain and backache pain, as well as muscular aches and strains. Because of the thinness of the patch, it is perceived as being more comfortable, more flexible, less obtrusive and is more acceptable to the patient. The backing 10 is rendered so translucent by infiltration of the hydrocolloidal gel that the patch is very inconspicuous on the skin. The entire thickness of the analgesic patch is about 0.34 mm.

The invention will be better understood by reference to the following examples:

EXAMPLES

Example Number	Percentage by Weight	Component
1	31.8	Glycerin
	0.2	Quatemium-15[1]
	21	Propylene Glycol
	1	Hydrocortisone
	25	Karaya
	21	HB Fuller 3120z[2]
2	31.8	Glycerin
	0.2	Quatemium-15[1]
	21.5	Propylene Glycol
	0.5	Hydrocortisone
	25	Karaya
	21	BF Goodrich 26171[3]
3	27.72	Glycerin
	0.64	Quatemium-15[1]
	24.5	Propylene Glycol
	0.5	Hydrocortisone
	24.64	Karaya
	21	BF Goodrich 26222[3]
4	27.72	Glycerin
	0.64	Quatemium-15[1]
	24.64	Propylene Glycol
	1	Hydrocortisone
	25	Karaya
	21	BF Goodrich 26171[3]
5	33	Glycerin
	18	Karaya
	9	34x[4]
	0.5	Hydrocortisone
	21.5	Propylene Glycol
	18	BF Goodrich 26171[3]
6	14	Methyl Salicylate
	4	Camphor
	6	Menthol
	76	BF Goodrich 26222[3]
7	29	Glycerin
	16	Polytec 31x[5]
	30	Propylene Glycol
	1	Hydrocortisone
	12	Lodex[6]
	4	H_2O (deionized)
	8	HB Fuller 3120z[2]
8	30.8	Glycerin
	15.4	Polytec 31x[5]
	22.8	Propylene Glycol
	8	Lidocaine
	12	Lodex[6]
	3	H_2O (deionized)
	8	HB Fuller 3120z[2]
9	30.8	Glycerin
	12	Karaya
	6.4	Lodex[6]
	8	34x[4]
	29.8	Propylene Glycol
	1	Capsicum
	12	Flexcryl 1615[7]
10	30.8	Glycerin
	12	Karaya
	5.4	Lodex[6]
	9	34x[4]
	25.8	Propylene Glycol
	12	HB Fuller 3120z[2]
	5	Benzocaine
11	31.4	Glycerin
	12.6	Karaya
	5.2	Lodex[6]
	8	34x[4]
	29.8	Propylene Glycol
	1	Hydrocortisone
	12	HB Fuller 3120z[2]

Example Number	Percentage by Weight	Component
12	14	Methyl Salicylate
	4	Camphor
	6	Menthol
	38	BF Goodrich 26171[3]
	38	BF Goodrich 26415[3]
13	14	Methyl Salicylate
	4	Camphor
	6	Menthol
	45	BF Goodrich 26415[3]
	31	BF Goodrich 26222[3]
14	17.4	Methyl Salicylate
	7.5	Camphor
	5.1	Menthol
	70	BF Goodrich 26415[3]
15	15.6	Methyl Salicylate
	6.8	Camphor
	4.6	Menthol
	25	BF Goodrich 26171[3]
16	19.8	Karaya
	36.6	Glycerin
	15.8	Methyl Salicylate
	2	Spearmint Oil
	25.8	HB Fuller 3120z[2]
17	19	Karaya
	37	Glycerin
	16	Methyl Salicylate
	2	Spearmint Oil
	13	BF Goodrich 26171[3]
	13	BF Goodrich 26415[3]
18	20	Karaya
	37	Glycerin
	8	Methyl Salicylate
	8	Trolamine Salicylate
	2	Spearmint Oil
	12.5	BF Goodrich 26415[3]
	12.5	BF Goodrich 26222[3]
19	15.6	Methyl Salicylate
	6.8	Camphor
	4.8	Menthol
	30	BF Goodrich 26334[3]
	43	BF Goodrich 26222[3]
20	15	Trolamine Salicylate
	10	Menthol
	34	BF Goodrich 26171[3]
	41	BF Goodrich 26222[3]
21	20.3	Methyl Salicylate
	6.6	Menthol
	32.5	BF Goodrich 26171[3]
	40.6	BF Goodrich 26222[3]
22	15	Methyl Salicylate
	10	Menthol
	29	BF Goodrich 26171[3]
	46	BF Goodrich 26222[3]
23	23	Karaya
	34	Glycerin
	11.5	Methyl Salicylate
	3	Menthol
	3	Camphor
	1.5	Spearmint Oil
	23	Avery AE259[8]
24	22.5	Karaya
	36	Glycerin
	16	Methyl Salicylate
	3	Spearmint Oil
	8	BF Goodrich 26222[3]
	14.5	BF Goodrich 26171[3]
	25	22.5 Karaya
	35.9	Glycerin
	11.8	Methyl Salicylate
	3.1	Camphor
	3.1	Menthol
	1.6	Spearmint Oil
	22	BF Goodrich 26415[3]

Example Number	Percentage by Weight	Component
26	24	Karaya
	34	Glycerin
	15	Methyl Salicylate
	2	Spearmint Oil
	12.5	BF Goodrich 26171[3]
	12.5	BF Goodrich 26334[3]
27	21	Karaya
	38	Glycerin
	15	Methyl Salicylate
	2	Spearmint Oil
	12	BF Goodrich 26415[5]
	12	BF Goodrich 26334[3]
28	23	Karaya
	37.5	Glycerin
	13.8	Methyl Salicylate
	1.7	Spearmint Oil
	12	BF Goodrich 26171[3]
	12	Aroset 1196[6]
29	22	Karaya
	36	Glycerin
	14.2	Methyl Salicylate
	1.8	Spearmint Oil
	3	Camphor
	11.5	Aroset 1196[9]
	11.5	BF Goodrich 26222[3]
30	22	Karaya
	35	Glycerin
	12	Methyl Salicylate
	3.2	Methol
	3.2	Camphor
	1.6	Spearmint Oil
	11	Avery AE259[8]
	12	BF Goodrich 26171[3]
31	54	Glycerin
	26	Karaya
	10	Flexcryl 1615[7]
	3.3	Eucalyptus Oil
	6.7	Menthol
32	23.5	Karaya
	33.5	Glycerin
	15.7	Methyl Salicylate
	2.8	Spearmint Oil
	9.1	BF Goodrich 26222[3]
	15.4	BF Goodrich 26171[3]
33	22.6	Karaya
	35.9	Glycerin
	6	Methyl Salicylate
	5.9	Trolamine Salicylate
	3.2	Camphor
	3.2	Menthol
	1.5	Spearmint Oil
	7.5	BF Goodrich 26222[3]
	14.2	BF Goodrich 26171[3]
34	22	Karaya
	35	Glycerin
	16	Methyl Salicylate
	4	Menthol
	6	Camphor
	2	Spearmint Oil
	9	BF Goodrich 26415[3]
	6	BF Goodrich 26171[3]
35	20	Karaya
	33.8	Glycerin
	0.2	Quaternium-15[1]
	16	Methyl Salicylate
	4	Menthol
	6	Camphor
	1.5	Spearmint Oil
	12	BF Goodrich 26222[3]
	6.5	BF Goodrich 26171[3]
36	54	Glycerin
	26	Karaya
	5	BF Goodrich 26222[3]
	5	BF Goodrich 26171[3]
	6.7	Menthol
	3.3	Eucalyptus Oil

Example Number	Percentage by Weight	Component
37	53	Glycerin
	25	Karaya
	9.5	Flexcryl 1615[7]
	8.4	Menthol
	4.1	Eucalyptus Oil
38	46.5	Glycerin
	8.4	Menthol
	4.1	Eucalyptus Oil
	26	Karaya
	15	Flexcryl 1615[7]
39	16.8	Menthol
	8.2	Eucalyptus Oil
	25	Avery AE259[8]
	34	Glycerin
	16	Karaya
40	54	Glycerin
	26	Karaya
	10	BF Goodrich 26222[3]
	6.7	Menthol
	3.3	Eucalyptus Oil
41	54	Glycerin
	26	Karaya
	10	BF Goodrich 26171[3]
	6.7	Menthol
	3.3	Eucalyptus Oil
42	54	Glycerin
	31	Karaya
	5	Flexcryl 1615[7]
	6.7	Menthol
	3.3	Eucalyptus Oil
43	54	Glycerin
	36	Karaya
	6.7	Menthol
	3.3	Eucalyptus Oil
44	49	Glycerin
	26	Karaya
	15	BF Goodrich 26171[3]
	6.7	Menthol
	3.3	Eucalyptus Oil
45	48	Glycerin
	24.5	Karaya
	15	BF Goodrich 26171[3]
	8.4	Menthol
	4.1	Eucalyptus Oil
46	49.3	Glycerin
	23.2	Karaya
	15	BF Goodrich 26334[3]
	8.4	Menthol
	4.1	Salicylic Acid
47	50	Glycerin
	25	Karaya
	15	BF Goodrich 26171[3]
	6.7	Menthol
	3.3	Eucalyptus Oil
48	47	Glycerin
	20.5	Karaya
	15	BF Goodrich 26415[3]
	11.7	Menthol
	5.8	Eucalyptus Oil
49	49.3	Glycerin
	23.2	Karaya
	15	BF Goodrich 26171[3]
	8.4	Menthol
	4.1	Eucalyptus Oil
50	47	Glycerin
	24.8	Karaya
	6.7	Menthol
	3.3	Eucalyptus Oil
	18.2	Aroset 1196[9]

Footnotes:

1 Quaternium-15 is a preservative comprising azoniaadamantane chloride by Dow Chemical of Palatine, IL.

2 HB Fuller 3120z is a residual vinyl acetate monomer resin emulsion in water by HB Fuller of Vadnais Heights, MN.

3 BF Goodrich 26171, 26222, 26334 and 26415 are acrylic ester copolymers of anionic emulsion adhesives by BF Goodrich of Brecksville, OH.

4 34x is an anionic polyacrylamide by Tecna Corporation of Belleville, NJ.

5 Polytec 31x is a non-ionic polyacrylamide by Tecna Corporation of Belleville, NJ.

6 Lodex is a carbohydrate comprising Malto Dextrin by American Maize-Product Company of Hammond IN.

7 Flexcryl 1615 is an adhesive of vinyl acetate / dioctylmaleate copolymer by Air Products and Chemical Inc of Allentown, PA.

8 Avery AE259 is an acrylic polymer fatex adhesive by Avery Chemical of Mill Hill, PA.

9 Aroset 1196 is an acrylic polymer adhesive by Ashland Chemical of Columbus, OH.

Many variations of the present invention within the scope of the appended claims will be apparent to those skilled in the art once the principles described herein are understood.

Claims

the internal portion of the hydrophilic hydrocolloidal reservoir which infiltrates the backing is solidified within the pores and interstices within the backing so that the reservoir and the backing are unified such that the backing acts as a storage location for the medication-containing reservoir,

the solidification of the gel reservoir is delayed until after the application thereof to the porous backing layer,

whereby medication within the reservoir migrates over time from within the backing through the lower coating layer and passes through the skin to provide sustained release of the medication into the body of the patient from the porous backing layer.

2. The adhesive patch of claim 1 wherein the porous backing layer is a nonwoven fabric comprising water insoluble polymeric fibers with spaces therebetween.

3. The adhesive patch of claim 2 wherein the nonwoven fabric comprises a mixture of polyester fibers and cotton fibers.

4. The adhesive patch of claim 1 wherein the reservoir comprises a hydrocolloidal dispersion of a hydrophilic natural or synthetic gel-forming polymer, a liquid humectant, an adhesive with said biomedically active medication dispersed therein and the natural or synthetic gel-forming polymer comprises a member selected from the group consisting of gum karaya, gum acacia, locust bean gum, guar gum, modified guar gum, maltodextrin, carboxymethyl cellulose, carboxypropyl cellulose, polyacrylamide and its cogeners, and polyacrylic acid.

5. The adhesive patch of claim 1 wherein the reservoir contains a humectant which comprises a polyhydric alcohol.

6. The adhesive patch of claim 5 wherein the polyhydric alcohol comprises a member selected from the group consisting of glycerol, propylene glycol, ethylene glycol, and sorbitol.

7. The adhesive patch of claim 1 wherein the adhesive is a resin emulsion adhesive.

8. The adhesive patch of claim 7 wherein the resin emulsion adhesive comprises a member selected from the group consisting of acrylate emulsion adhesive, an acrylic ester copolymer, a vinyl acetate resin, and copolymer of vinyl acetate and dioctyl maleate.

9. The adhesive patch of claim 1 wherein the medication comprises one or more of the following: trolamine salicylate, methyl salicylate, menthol, camphor, eucalyptus oil, spearmint oil, hydrocortisone, benzocaine, lidocaine, ibuprofen, salicylic acid, and capsicum.

10. The non-occlusive adhesive patch of claim 1 wherein the internal portion of the adhesive reservoir penetrates the porous backing layer to a depth of about at least one-fourth the thickness of the backing layer.

11. The non-occlusive adhesive patch of claim 1 wherein the internal portion of the adhesive reservoir penetrates the porous backing layer to a depth of about nine-tenths the thickness of the backing layer.

12. A method of forming a non-occlusive medication-containing adhesive patch to be applied to the skin for releasing a medication into the body of a patient comprising,

providing a porous backing layer of flexible water insoluble polymeric sheet material,

forming a dispersion of a polymer comprising an adhesive and a biomedically active medication to provide a pressure-sensitive hydrocolloidal gel reservoir,

expelling the hydrocolloidal gel onto the backing layer while maintaining the dispersion in a chilled condition,

the hydrocolloidal gel being chilled to a temperature effective to maintain the dispersion sufficiently fluid when applied to the backing layer to penetrate the backing layer to a depth of at least about one-fourth the thickness of the backing layer,

forming the hydrocolloidal gel into a coating on the backing, said coating having a flat, exposed, pressure-sensitive surface for bonding to the skin,

said pressure-sensitive hydrocolloidal gel reservoir thereby having two portions including:

(a) an external coating layer with said exposed surface for bonding to the skin, and

(b) an internal portion infiltrated within the pores of the backing layer, and allowing the hydrocolloidal gel to cure in place upon and within the porous backing layer.

13. The method of claim 12 wherein the curing is accelerated by heating the hydrocolloidal gel after the gel has infiltrated into the pores of the backing layer.

14. The method of claim 12 wherein the coating of the hydrocolloidal gel is formed by a knife coater to provide said pressure-sensitive surface for bonding to the skin.

15. The method of claim 14 wherein a sheet of a liner paper is applied to the pressure-sensitive surface of the hydrocolloidal gel coating to protect the same during shipment and storage.

16. The method of claim 12 wherein the dispersion is formed continuously within a processing mixer and is then applied as a continuous stream on a moving strip of said backing layer and a web of liner paper having a release surface is applied continuously to the exposed pressure-sensitive surface of the hydrocolloidal gel coating to protect the coating during shipment and storage, and said adhesive patches are cut therefrom.

17. The method of claim 16 wherein the hydrocolloidal gel reservoir is infiltrated into the porous backing layer to a depth of between about one-fourth and nine-tenths the thickness of the backing layer and is solidified within the pores of the backing layer to unify the backing layer and the coating such that the backing layer acts as a storage location for the medication-containing hydrocolloidal gel reservoir.

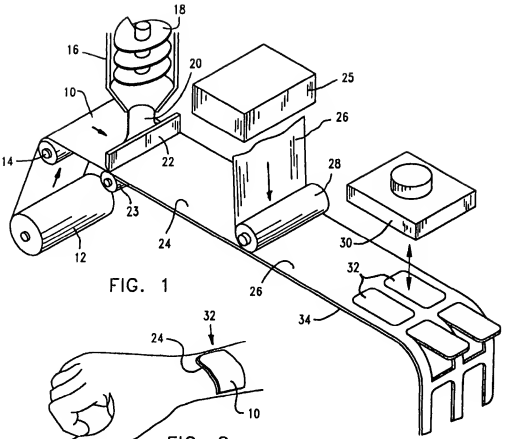

FIG. 1

FIG. 2

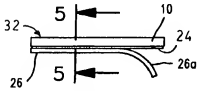

FIG. 4

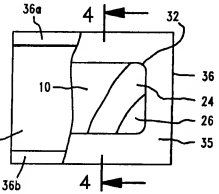

FIG. 3

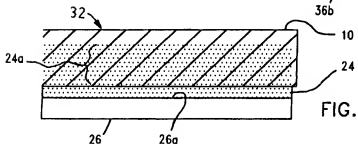

FIG. 5

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(54) **Pharmazeutische und/oder kosmetische Zubereitung sowie die Verwendung einer derartigen Zubereitung.**

(57) Es wird eine pharmazeutische und/oder kosmetische Zubereitung zur topischen Anwendung beschrieben, wobei die Zubereitung neben mindestens einem Wirkstoff desweiteren ein Trägermaterial für den Transport des Wirkstoffes in die Haut aufweist. Die Zubereitung enthält als Wirkstoff Linolsäure und/oder mindestens ein Linolsäurederivat.

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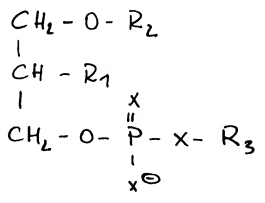
Die vorliegende Erfindung betrifft eine pharmazeutische und/oder kosmetische Zubereitung mit den Merkmalen des Oberbegriffs des Patentanspruches 1 sowie die Verwendung einer derartigen Zubereitung.

Pharmazeutische und/oder kosmetische Zubereitungen zur topischen Anwendung sind seit langem bekannt. In den üblichen Salben, Cremes, Lösungen, Emulsionen oder Suspensionen, die in der Regel rein örtlich auf der Oberfläche des behandelten Hautbereiches wirken, sind auch solche Systeme bekannt, die ein Trägermaterial aufweisen, wobei das Trägermaterial sicherstellen soll, daß der in der Zubereitung enthaltende Wirkstoff in die Haut bzw. durch die Hautbarriere transportiert wird. So beschreibt beispielsweise die DE PS 3829899 derartige pharmazeutische Zubereitungen, wobei hier als Wirkstoff Phospholipidderivate der nachfolgenden allgemeinen Formel (I) enthalten sind.



(Formel I)

In der Formel I bedeuten R_1 Wasserstoff eine niedrige aliphatische Alkylgruppe mit 1 bis 12 Kohlenstoffatomen, eine Alkoxygruppe mit etwa 6 bis 30 Kohlenstoffatomen, eine Alkylcarbonyloxidgruppe mit etwa 6 bis 30 Kohlenstoffatomen, ein Furanosyl- oder Pyranosylrest mit 4 bis etwa 50 Kohlenstoffatomen und insgesamt bis zu 10 glykosidisch verknüpfte Furanose- und/oder Pyranoseringen, R_2 eine aliphatische Alkylgruppe mit etwa 6 bis 30 Kohlenstoffatomen oder eine aliphatische Alkylcarbonylgruppe mit etwa 6 bis 30 Kohlenstoffatomen, X Sauerstoff, Schwefel oder die Iminogruppe, R_3 der Rest eines Aminoalkohols der allgemeinen Formel R_4 - N (R_5 R_6), wobei R_4 eine ggf einen Carboxylrest tragende Alkylen-Brückengruppe mit 1 bis etwa 12 Kohlenstoffatomen, R_5 und R_6 unabhängig voneinander Wasserstoff oder eine niedrige Alkylgruppe mit einem bis 4 Kohlenstoffatomen sind.

Der vorliegenden Erfindung liegt die Aufgabe zugrunde, eine neue pharmazeutische und/oder kosmetische Zubereitung zur topische Anwendung zur Verfügung zu stellen, die eine besonders hohe pharmazeutische und/oder kosmetische Wirksamkeit besitzt.

Diese Aufgabe wird erfindungsgemäß durch eine pharmazeutische und/oder kosmetische Zubereitung mit den kennzeichnenden Merkmalen des Patentanspruchs 1 gelöst.

Die erfindungsgemäße pharmazeutische und/oder kosmetische Zubereitung, die zur topischen Anwendung eingesetzt wird, weist, wie der vorbeschriebene Stand der Technik, neben mindestens einem Wirkstoff desweiteren ein Trägermaterial auf, wobei das Trägermaterial sicherstellt, daß der Wirkstoff nicht lokal auf der Hautoberfläche verbleibt sondern in die Haut bzw. durch die Hautbarriere transportiert wird. Hierbei enthält die erfindungsgemäße pharmazeutische und/oder kosmetische Zubereitung als Wirkstoff Linolsäure und/oder mindestens ein Linolsäurederivat.

Die erfindungsgemäße pharmazeutische und/oder kosmetische Zubereitung weist eine Reihe von Vorteilen auf. So ist zunächst festzuhalten, daß eine derartige Zubereitung eine besonders hohe pharmazeutische und/oder kosmetische Wirksamkeit, insbesondere auch bei Hauterkrankungen und vorzugsweise bei unreiner Haut, Akne und den damit verbundenen Begleiterscheinungen, besitzt, was darauf zurückgeführt wird, daß der in der erfindungsgemäßen Zusammensetzung enthaltene Wirkstoff, d.h. die Linolsäure oder das mindestens eine Linolsäurederivat, sehr schnell in die Haut eindringt bzw. die Hautbarriere überwindet, so daß nach Auftragen der erfindungsgemäßen Zubereitung sehr schnell hohe Konzentrationen an Wirkstoff zu dem erkrankten Bereich gelangen. Bedingt dadurch, daß die erfindungsgemäße Zubereitung topisch, d.h. somit über die Haut, appliziert wird, sind die von der erfindungsgemäßen pharmazeutischen und/oder kosmetischen Zusammensetzung hervorgerufenen Nebenwirkungen minimalisiert, was für solche bekannten Produkte nicht zutrifft, die beispielsweise oral oder durch Injektion appliziert werden. Auch zeigte sich überraschend, daß der in der erfindungsgemäßen pharmazeutische und/oder kosmeti-sche Zubereitung enthaltene Wirkstoff Linolsäure und/oder ein entsprechendes Linolsäurederivat eine hohe pharmazeutische

und/oder kosmetische Wirksamkeit besitzt, insbesondere dann, wenn die erfindungsgemäße pharmazeutische und/oder kosmetische Zubereitung zur Therapie und/oder Prophylaxe von unreiner Haut, Akne, Pickel, Pusteln, und/oder den damit verbundenen Begleiterscheinungen eingesetzt wird. So konnte beispielsweise festgestellt werden, daß bereits nach wenigen topischen Anwendungen der erfindungsgemäßen Zubereitung die auf der unreinen Haut vorhandenen Pickel, Quaddeln, Pusteln, Entzündungen oder Mitessern deutlich zurückgingen, so daß bereits nach wenigen Tagen der Anwendung der erfindungsgemäßen Zubereitung eine reine, geschmeidige und glatte Haut resultierte. Auch bei Akne, insbesondere bei schwierigen und langanhaltenden Akne-Erkrankungen, ließ sich durch Anwendung der erfindungsgemäßen Zubereitung innerhalb von relativ kurzen Behandlungszeiträumen, die abhängig von dem Erkrankungsgrad zwischen 2 Wochen und etwa 6 Wochen variierten, eine deutliche Besserung und in den überwiegenden Fällen eine anhaltende Heilung, erzielen, was im Vergleich mit herkömmlichen Akne-Mitteln nicht möglich war. Darüber hinaus wurde die mit der erfindungsgemäßen pharmazeutischen und/oder kosmetischen Zubereitung behandelten Hautbereiche durch die Behandlung deutlich geschmeidiger und elastischer, was bei den behandelten Personen ein Eindruck von gesunder Haut hervorrief.

Eine erste Ausführungsform der erfindungsgemäßen Zubereitung sieht vor, daß die Zubereitung als Trägermaterial Liposome aufweist. Hierbei werden unter dem Begriff Liposom im Sinne der vorliegenden Anmeldung solche Vesikel verstanden, die abhängig von ihrem jeweiligen Herstellungsverfahren unilamellare, oligolamellare, multilamellare Vesikel oder fusionierte Körper darstellen. Derartige Liposome besitzen eine Hohlkörperstruktur, wobei der Mantel des Hohlkörpers von einer Membran gebildet wird. Ihr Durchmesser variiert zwischen ca. 15 nm und 3.500 nm, insbesondere zwischen 100 nm und 300 nm.

Ähnlich wie die biologischen Zellen können Liposome in ihren vesikulären Innenbereichen wasserlösliche Substanzen und/oder in ihrer Membran lipophile Substanzen speichern, wobei im Falle der erfindungsgemäßen Zubereitung die Linolsäure bzw. das Linolsäurederivat aufgrund seiner Lipophilie in den die Liposome bildenden Membranen gespeichert ist. Als membranbildende Substanz kommen synthetische oder natürliche Polymere in Frage, so zum Beispiel Polyacrylate, Polyester und/oder polymere Cellulosederivate.

Eine besonders geeignete Ausführungsform der erfindungsgemäßen Zubereitung sieht vor, daß die Liposome aus mindestens einem Phospholipid gebildet sind. Als Ausgangspunkt für derartige Phospholipide, die vorzugsweise ein Gemisch von Phospholipiden darstellen, dienen die natürlichen pflanzlichen oder tierischen Lecithinen, aus denen durch Extraktion und anschließender Reinigung nach den an sich bekannten Verfahren derartige, die Liposome bildende Phospholipide gewonnen werden. Vorzugsweise werden diese Phospholipide bzw. Phospholipidgemische aus Eiern, Ölsaaten sowie Ölfruchten, wie beispielsweise Kokosnuß, Kopra, Palmenkernen, Erdnüssen, Raps, Sonnenblumenkernen, Ölpalmen und/oder Oliven isoliert, und, wie bereits vorstehend erwähnt, nach entsprechender Reinigung und Konzentrierung zu den Liposomen verarbeitet.

Eine besonders geeignete Ausführungsform der erfindungsgemäßen Zubereitung weist Liposome auf, die aus einem Phospholipid bzw. Phospholipidgemisch gebildet werden, wobei das hierfür eingesetzte Phospholipid bzw. Phospholipidgemisch nach den an sich bekannten Verfahren aus pflanzlichen Ausgangsmaterialien, insbesondere aus Sonnenblumen oder Soja-Bohnen, isoliert wird. Somit stellen diese Liposome die zuvor beschriebenen Vesikel dar, deren Membran aus pflanzlichen Phospholipiden, insbesondere aus Soja-Phospholipiden oder Sonneblumen-Phospholipiden, besteht.

Insbesondere dann, wenn die erfindungsgemäße Zusammensetzung Liposome aufweist, die aus Soja-Phospholipiden gebildet werden, die eine Konzentration zwischen 70 Gew.% und 100 Gew.% 1,2-Diacylglycero-3-Phosphocholin [(3-sn-Phosphatidyl)cholin, Soja] enthalten, weist eine derartige erfindungsgemäße Zubereitung eine hervorragende pharmazeutische und/oder kosmetische Wirksamkeit auf. Dies hängt damit zusammen, daß derartige, an Phosphocholin reiche und somit reproduzierbar herstellbare Liposome ein hohes Speichervermögen für Linolsäure und/oder Linolsäurederivate aufweisen, so daß sie dementsprechend auch reproduzierbar mit Linolsäure bzw. Linolsäurederivate beladen werden können. Dies wiederum hat zufolge, daß beim Auftragen von gleichen Mengen der erfindungsgemäßen Zubereitung auf die Haut stets gleiche Mengen an Linolsäure bzw. Linolsäurederivaten aufgebracht und über ein derartiges Trägermaterial in die Haut bzw. durch die Hautbarriere transportiert werden, was wiederum die Reproduzierbarkeit der durch die erfindungsgemäße Zubereitung erzielten Heilerfolge sicherstellt. Von daher wird eine derartige Ausführungsform der erfindungsgemäßen Zubereitung insbesondere dann bevorzugt, wenn die erfindungsgemäße Zubereitung im eingangs genannte Sinne zur pharmazeutische oder kosmetische Behandlung von unreiner Haut, Pickeln, Pusteln, Quaddeln, sowie Akne und deren Begleiterscheinungen eingesetzt wird.

Bezüglich des Anteils an Trägermaterial, das die erfindungsgemäße Zubereitung aufweist, ist allgemein festzuhalten, daß dieser Anteil so hoch sein muß, daß ein einwandfreier Transport der Zubereitung in die

Haut bzw. durch die Hautbarriere sichergestellt ist. Vorzugsweise weist die erfindungsgemäße Zubereitung zwischen 5 Gew.% und 50 Gew.%, insbesondere zwischen 15 Gew.% und 30 Gew.%, Trägermaterial und vorzugsweise die zuvor beschriebenen phospholipidischen Trägermaterialien auf, wobei sich die zuvor angegebenen prozentualen Gewichtsangaben auf die anwendungsfertige Zubereitung beziehen.

Um bei der topischen Anwendung der erfindungsgemäßen Zubereitung die Aufnahme der Zubereitung durch die Haut zu erleichtern, besitzt die erfindungsgemäße Zubereitung vorzugsweise eine flüssige bis halbfeste Konsistenz d. h. die erfindungsgemäße Zubereitung ist insbesondere als Gel oder Flüssigkeit formuliert. Eine derartige gelartige bzw. flüssige Formulierung der erfindungsgemäßen Zubereitung wird insbesondere dadurch erreicht, daß die erfindungsgemäße Zubereitung neben dem Trägermaterial und dem Wirkstoff (Linolsäure und/oder Linolsäurederivat) Wasser und/oder ein nicht toxisches Lösungsmittel, insbesondere einen wasserlöslichen Alkohol, aufweist. Hierfür eignen sich insbesondere als wasserlöslicher Alkohol Ethanol, Propanol-1 und Propanol-2, und/oder Propylenglykol. Unter dem Begriff Wasser fallen im Rahmen der vorliegenden Anmeldung alle wäßrigen Systeme, wie insbesondere gereinigtes Wasser, destilliertes Wasser, entioniertes Wasser sowie wäßrige Salzlösungen, vorzugsweise physiologische Kochsalzlösungen, oder Puffersysteme, vorzugsweise Phosphatpuffer.

Vorstehend ist im Zusammenhang mit der erfindungsgemäßen Zubereitung dargelegt worden, daß die erfindungsgemäße Zubereitung Linolsäure und/oder mindestens ein Linolsäurederivat aufweist. Hierbei bestehen grundsätzlich mehrere, nachfolgend noch näher erläuterte Möglichkeiten, um die erfindungsgemäße Zubereitung mit Linolsäure bzw. dem mindestens einen Linolsäurederivat zu versehen.

So sieht eine erste Möglichkeit vor, daß der Wirkstoff (Linolsäure und/oder das Linolsäurederivat) physikalisch an das Trägermaterial gebunden ist. Werden als Trägermaterial beispielsweise die zuvor beschriebenen Liposome, insbesondere die phospholipidischen Liposome, verwendet, so ist vorzugsweise der Wirkstoff in die Membran bzw. in die phospholipidische Membran, die als äußere Hülle die kugelförmigen Vesikel umschließt, eingelagert. Zusätzlich oder anstelle einer derartigen Einlagerung besteht desweiteren noch die Möglichkeit, daß der Wirkstoff als wäßrige Dispersion innerhalb der Vesikel vorhanden ist.

Anstelle der zuvor beschriebenen physikalischen Anbindung der Linolsäure und/oder des mindestens einen Linolsäurederivates an das Trägermaterial sieht die zweite Möglichkeit der erfindungsgemäßen Zubereitung vor, daß hierbei der Wirkstoff (Linolsäure und/oder das mindestens eine Linolsäurederivat) chemisch an das Trägermaterial gebunden ist.

Bei der dritten Möglichkeit der erfindungsgemäßen Zubereitung sind die zuvor beschriebenen ersten und zweiten Möglichkeiten miteinander kombiniert, d.h. diese dritte Möglichkeit sieht vor, daß die Linolsäure und/oder das mindestens eine Linolsäurederivat sowohl chemisch als auch physikalisch an das Trägermaterial angebunden sind.

Eine Weiterbildung der zuvor beschriebenen dritten Möglichkeit verwendet als Trägermaterial ein Phospholipid, wobei eine derartige, besonders geeignete Ausführungsform der erfindungsgemäßen Zubereitung dann eine chemisch und auch physikalisch an das Phospholipid angebundene Linolsäure und/oder ein chemisch und physikalisch an das Phospholipid angebundenes Linolsäurederivat aufweist. Um eine derartige chemische Anbindung der Linolsäure bzw. des mindestens einen Linolsäurederivates an das Phospholipid zu erreichen, ist das als Trägermaterial verwendete Phospholipid mit der Linolsäure und/oder mit dem mindestens einen Linolsäurederivat acyliert. Hierbei zeichnet sich eine derartige Ausführungsform durch eine besonders hohe pharmazeutische und/oder kosmetische Wirksamkeit aus.

Selbstverständlich ist es möglich, auch solche Ausführungsformen der erfindungsgemäßen Zubereitung zu verwenden, die als Wirkstoff ein mit Linolsäure und/oder dem Linolsäurederivat acyliertes Phospholipid enthält, wobei eine derartige Ausführungsform dann unter Berücksichtigung der zuvor beschriebenen drei Möglichkeiten den Wirkstoff (Linolsäure und/oder Linolsäurederivat) nur chemisch an dem Phospholipid gebunden beinhaltet.

Vorstehend ist im Zusammenhang mit dem Trägermaterial beschrieben worden, daß das Trägermaterial ein Phospholipid bzw. ein Phospholipidgemisch umfaßt. Hierunter fallen insbesondere die bereits vorstehend genannten und aus pflanzlichen und/oder tierischen Lecithinen isolierten Phospholipide, wobei insbesondere in der erfindungsgemäßen Zubereitung 1,2-Diacylglycero-3-Phosphocholin[(3-sn-Phosphatidyl)cholin] allein oder in Mischung mit anderen Phospholipiden als Trägermaterial enthalten ist. Als weitere Phospholipide, die als Trägermaterial in der erfindungsgemäßen Zusammensetzung enthalten sein können, sind vorzugsweise 1,2-Diacylglycero-3-Phosphoethanolamin, 1,2-Diacylglycero-3-Phosphoinositol, 1,2-Diacylglycero-3-Phosphoserin, 1,2-Diacylglycero-3-Phosphoglycerol und 1,2-Diacylglycero-3-Phosphat jeweils allein oder in Mischung zu nennen.

Weist die erfindungsgemäße Zusammensetzung die zuvor genannten und mit Linolsäure und/oder dem Linolsäurederivat acylierten Phospholipide auf, so ist zwischen der Acylierung mit Linolsäure bzw. dem Linolsäurederivat in 1-Stellung, der Acylierung mit Linolsäure und/oder dem Linolsäurederivat in 1- und 2-

Stellung sowie der Acylierung mit Linolsäure und/oder dem Linolsäurederivat in 2-Stellung zu unterscheiden. Dementsprechend weist die erfindungsgemäße Zubereitung 1-Linolylglycero-3-Phosphate, 1,2-Dilinolylglycero-3-Phosphate und/oder 2-Linolylglycero-3-Phosphate der vorstehend genannten Art auf.

Eine besonders geeignete Ausführungsform der erfindungsgemäßen Zubereitung enthält mindestens ein mit Linolsäure oder einem Linolsäurederivat acyliertes Phospholipid bzw. Phospholipidgemisch der vorstehend genannten Art, wobei mindestens 60 Gew.% der Acylreste Linolsäure und/oder Linolsäurederivate darstellen.

Bei einer besonders geeigneten und sehr wirksamen Weiterbildung der zuvor beschriebenen erfindungsgemäßen Zubereitung enthält die Zubereitung ein solches acyliertes Phospholipidgemisch, bei dem die in dem Gemisch enthaltenen Acylreste zu

61 - 73 Gew.% aus dem Linolsäurerest,
10 - 14 Gew.% aus dem Palmitinsäurerest,
8 - 12 Gew.% aus dem Ölsäurerest,
4 - 6 Gew.% aus dem Linolensäurerest,
3 - 5 Gew.% aus dem Stearinsäurerest und
bis zu 2 Gew.% aus anderen Fettsäureresten

bestehen.

Insbesondere dann, wenn die zuvor beschriebenen vorteilhafte Weiterbildung der erfindungsgemäßen pharmazeutischen Zubereitung 15 - 30 Gew.%, bezogen auf die anwendungsfertige Zubereitung, eines Phospholipidgemisches, mit einem Gehalt von 70 bis 100 Gew.% (bezogen auf das Phospholipidgemisch) 1,2-Diacylglycero-3-Phosphocholin beinhaltet, wobei das in dem Phospholipidgemisch enthaltene Acylrestegemisch mindestens 60 Gew.% Linolsäurereste enthält, weist eine derartige Ausführungsform der erfindungsgemäßen Zubereitung hervorragende pharmazeutische Wirkungen in bezug auf die Behandlung von Akne und der damit verbundenen Begleiterkrankungen auf. Die dann noch verbleibenden maximal 40 Gew.% Acylreste umfassen insbesondere den Palmitinsäurerest, den Ölsäurerest, den Linolsäurerest und/oder den Stearinsäurerest, vorzugsweise in den zuvor angegebenen Massenverhältnissen.

Wie bereits vorstehend erwähnt ist, kann das in der erfindungsgemäßen Zubereitung vorgesehene Phospholipid ein Phospholipidgemisch sein. Hierfür kommen insbesondere die bereits vorstehend genannten 1,2-Diacylglycero-3-phosphat (1,2-Diacylglycero-3-phosphoethanolamin, 1,2-Diacylglycero-3-phosphoinositol, 1,2-Diacylglycero-3-phosphoserin, 1,2-Diacylglycero-3-phosphoglycerol und/oder 1,2-Diacylglycero-3-phosphat) in Frage, wobei vorzugsweise bis zu 30 Gew.% der vorstehend genannten 1,2-Diacylglycero-3-phosphate in dem Phospholipidgemisch enthalten sind, während eine derartige Ausführungsform der erfindungsgemäßen Zubereitung dann mindestens 70 Gew.% des 1,2-Diacylglycero-3-phosphocholin aufweist. Hierbei beziehen sich die zuvor genannten prozentualen Massenangaben auf die Gesamtmasse des in der erfindungsgemäßen Zubereitung enthaltenen Phospholipidgemisches, das, wie bereits vorstehend ausgeführt ist, vorzugsweise in der anwendungsfertigen Zubereitung zu 5 bis 50 Gew.%, bezogen auf die Masse der fertigen Zubereitung, als Trägermaterial enthalten ist.

Eine andere, ebenfalls besonders geeignete Ausführungsform der erfindungsgemäßen Zubereitung beinhaltet als Phospholipid ein 1,2-Diacylglycero-3-phosphocholin, bei dem der 1-Acylrest

45 - 61 Gew.% Linolsäurereste,
19 - 26 Gew.% Palmitinsäurerest,
8 - 12 Gew.% Ölsäurereste,
4 - 6 Gew.% Linolensäurereste,
6 - 9 Gew.% Stearinsäurereste und/oder
2 Gew.% andere Fettsäurereste

umfaßt. Um hierbei sicherzustellen, daß in einer derartigen Zubereitung auch die erforderliche Menge an Linolsäure bzw. dem Linolsäurederivat enthalten ist, kann einer Ausführungsform entweder freie Linolsäure bzw. Linolsäurederivat zugesetzt werden oder der in 2-Stellung befindliche Acylrest entsprechend mit Linolsäure acyliert sein, wobei vorzugsweise solche 1,2-Diacylglycero-3-phosphocholine eingesetzt werden, bei denen der 1-Acylrest die vorstehend angegebene Zusammensetzung aufweist und der 2-Acylrest

77 - 85 Gew.% Linolsäurereste,
1 - 2 Gew.% Palmitinsäurereste,
8 - 12 Gew.% Ölsäurereste,
4 - 6 Gew.% Linolensäurereste,
0 - 1 Gew.% Stearinsäurereste und/oder
2 Gew.% andere Fettsäurereste

umfaßt.

Bezüglich der Konzentration an Linolsäure und/oder dem mindestens einen Linolsäurederivat, die bzw. das in der erfindungsgemäßen Zubereitung als Wirkstoff chemisch und/oder physikalisch am Trägermaterial gebunden enthalten ist, ist festzuhalten, daß diese Konzentration an freier bzw. gebundener Linolsäure und/oder an freiem und/oder gebundenem Linolsäurederivat zwischen 1 Gew.% und 30 Gew.%, vorzugsweise zwischen 3 Gew.% und 18 Gew.%, variiert. Wird die erfindungsgemäße Zubereitung als kosmetische Zubereitung eingesetzt, weist eine derartige kosmetische Zubereitung die Linolsäure bzw. das Linolsäurederivat in Konzentrationen auf, die vorzugsweise zwischen 1 Gew.% und 8 Gew.% variieren, während entsprechende pharmazeutische Zubereitungen Wirkstoffkonzentrationen enthalten, die insbesondere zwischen 15 Gew.% und 30 Gew.% liegen. Hierbei beziehen sich die zuvor wiedergegebenen prozentualen Massenangaben an Linolsäure bzw. dem mindestens einen Linolsäurederivat auf die anwendungsfertige Zubereitung.

Wie bereits vorstehend ausgeführt ist, besitzt die erfindungsgemäße Zubereitung vorzugsweise eine gelartige oder flüssige Konsistenz. Dies bedeutet, daß die erfindungsgemäße Zubereitung insbesondere als Gel, Lösung, Lotion, Salbe, Creme, Spray und/oder Aerosol formuliert wird. Hierbei können übliche Hilfsstoffe, wie beispielsweise Konsistenzgeber (CMC, Gabapol, Alginate, Xantan) und/oder dermatologische Konservierungsmittel zugesetzt werden, wobei jedoch darauf zu achten ist, daß Stoffe, die die Fettproduktion anregen, die die Haut abdecken oder die selbstfettend wirken, vermieden werden sollen. Darüber hinaus sollte das Konservierungsmittel selbst nicht penetrieren, wobei als bevorzugte Konservierungsstoffe somit wasserlösliche Alkohole, insbesondere Propylenglykol, in der erfindungsgemäßen Zubereitung enthalten ist.

Eine bevorzugte gelartige und insbesondere liposomale Ausführungsform der erfindungsgemäßen Zubereitung weist dann zwischen

30 Gew.% und 93 Gew.% Wasser,

0 Gew.% und 20 Gew.% der zuvor genannten Lösungsmittel,

1 Gew.% und 30 Gew.% Linolsäure und/oder dem mindestens einen Linolsäurederivat sowie

5 Gew.% und 50 Gew.% Trägermaterial, insbesondere die zuvor genannten Phospholipide,

auf.

Hierbei sind in einer derartigen gelartigen Zubereitung als Lösungsmittel insbesondere die bereits genannten Alkohole enthalten. Diese gelartige Zubereitung besitzt zudem noch den Vorteil, daß sie in ihrer Anwendung besonders einfach zu handhaben ist.

Eine typische flüssige und insbesondere auch liposomale Ausführungsformen der erfindungsgemäßen Zubereitung weist zwischen

69 Gew.% und 94 Gew.% Wasser,

0 Gew.% und 20 Gew.% Lösungsmittel,

1 Gew.% und 30 Gew.% Linolsäure und/oder dem mindestens einen Linolsäurederivat sowie

5 Gew.% und 30 Gew.% Trägermaterial, insbesondere die zuvor genannten Phospholipide,

auf.

Um bei der zuvor beschriebenen flüssigen Ausführungsform der erfindungsgemäßen Zubereitung eine lange Haltbarkeit sicherzustellen, empfiehlt es sich, hier der Zubereitung Lösungsmittel zuzusetzen, insbesondere der Zubereitung die vorstehend genannten wasserlöslichen Alkohole zuzugeben. Die nur Wasser enthaltenden flüssigen Formulierungen sind dann vorzuziehen, wenn hiermit Patienten behandelt werden sollen, bei denen Lösungsmittel und insbesondere auch wasserlösliche Alkohole Hautirritationen hervorrufen. Dies trifft jedoch nur auf eine geringe Zahl von besonders empfindlichen Patienten zu.

Um die notwendige Sterilität bei solchen flüssigen Formulierungen der erfindungsgemäßen Zubereitung, die keine organischen Lösungsmittel und insbesondere auch keine Alkohole enthalten, sicherzustellen, sieht eine Weiterbildung dieser Ausführungsform vor, daß die flüssige Zubereitung in gasdichten Ampullen abgepackt ist. Hierbei weist dann die gasdichte Ampulle so viel von der flüssigen erfindungsgemäßen Zubereitung auf, die ausreicht, um die erfindungsgemäße Zubereitung einmalig anzuwenden.

Eine weitere, ebenfalls bevorzugte Ausführungsform der erfindungsgemäßen Zubereitung sieht vor, daß die Zubereitung eine erste Komponente sowie eine hiervon getrennt verpackte zweite Komponente umfaßt. Mit anderen Worten sind bei dieser Ausführungsform der erfindungsgemäßen Zubereitung zwei, während der Lagerung getrennt gehaltene Komponenten vorhanden, wobei diese beiden Komponenten unmittelbar vor der Anwendung der erfindungsgemäßen Zubereitung von dem jeweiligen Benutzer miteinander vermischt werden. Hierbei enthält die erste Komponente ein Lösungsmittel, mindestens einen Elektrolyten und/oder Wasser, wobei es sich bei dem Lösungsmittel um die bereits vorstehend genannten Lösungsmittel handelt. Als Elektrolyten kann jeder, im medizinischer Bereich eingesetzter Elektrolyt eingesetzt werden, vorzugsweise jedoch handelt es sich bei dem Elektrolyten um eine physiologische Kochsalzlö-sung. Die zweite Komponente, die, wie bereits vorstehend erwähnt ist, von der ersten Komponente getrennt verpackt

ist, enthält bei dieser Ausführungsform das Trägermaterial, die Linolsäure und/oder das Linolsäurederivat sowie ggf. ein Lösungsmittel der vorstehend genannten Art. Der Zusatz des Lö-sungsmittels zur zweiten Komponente weist den Vorteil auf, daß dann bei einer derartigen Abwandlung der erfindungsgemäßen Zubereitung zwei flüssige Komponenten vorliegen, die unmittelbar vor der Anwendung sehr leicht miteinander vermischt werden können.

Um bei der zuvor beschriebenen vorteilhaften Ausführungsform der erfindungsgemäßen Zubereitung, die zwei Komponenten vorsieht, den erforderlichen pH-Wert einzustellen, ist bei einer Weiterbildung dieser Ausführungsform eine der beiden Komponenten, insbesondere die erste Komponente, mit einem geeigneten pH-Regulator versehen. Hierbei handelt es sich dann vorzugsweise um ein wäßriges Puffersystem oder um eine entsprechende Lauge, wobei die pH-Werte der anwendungsfertigen und vermischten Zubereitung zwischen 5,5 und 8, vorzugsweise zwischen 6,5 und 7,5, variieren.

Bezüglich des Trägermaterials bei der zuvor beschriebenen Ausführungsform, die zwei Komponenten beinhaltet, ist festzuhalten, daß hier die Trägermaterialien eingesetzt werden können, wie diese vorstehend im Zusammenhang mit anderen Ausführungsformen der erfindungsgemäßen Zubereitung beschrieben sind. Insbesondere wird hierfür jedoch eine zweite Komponente verwendet, die aus Linolsäure und/oder dem mindestens einen Linolsäurederivat besteht, wobei die Linolsäure bzw. das Linolsäurederivat physikalisch und/oder chemisch an das phospholipidische Trägermaterial gebunden ist. Insbesondere dann, wenn ein mit Linolsäure bzw. dem Linolsäurederivat acyliertes Phospholipid und vorzugsweise das zuvor quantitativ beschriebene spezielle acylierte 1,2-Diacylglycero-3-Phosphocholin in der zweiten Komponenten enthalten ist, weist eine derartige Weiterbildung der zuvor beschriebenen Ausführungsform der erfindungsgemäßen Zubereitung hohe pharmazeutische und/oder kosmetische Wirksamkeiten auf.

Bezüglich der Konzentrationen der zuvor beschriebenen besonders geeigneten Ausführungsformen der erfindungsgemäßen Zubereitung, die die erste und zweite, unmittelbar vor der Anwendung zu vermischenden Komponenten aufweist, ist festzuhalten, daß hierbei insbesondere die erste Komponente zwischen

50 Gew.% bis 80 Gew.% Wasser sowie
zwischen 0 Gew.% und 20 Gew.% Lösungsmittel

und die zweite Komponente zwischen

3 Gew.% und 15 Gew.% des mit Linolsäure oder dem mindestens einen Linolsäurederivat acylierten phospholipidischen Trägermaterials sowie
0 Gew.% bis 15 Gew.% Lösungsmittel

aufweist.

Eine Weiterbildung dieser zuvor beschriebenen Ausführungsform sieht vor, daß das in der ersten Komponente enthaltene Wasser (50 Gew.% bis 80 Gew.%) durch eine wäßrige Elektrolytlösung und vorzugsweise durch eine wäßrige physiologische Kochsalzlösung (0,5 Gew.% bis 2,5 Gew.% Kochsalz) vollständig oder teilweise ersetzt ist. Eine derartige, elektrolythaltige Weiterbildung zeichnet sich durch eine hohe Sterilität und eine besonders lange Haltbarkeit auf.

Desweiteren können in der ersten Komponente und/oder der zweiten Komponente noch übliche Hilfsstoffe, wie beispielsweise pH-Regulatoren, Puffersysteme, Emulgatoren und/oder Verdickungsmittel, enthalten sein.

Wie bereits vorstehend wiederholt ausgeführt ist, wird die erfindungsgemäße Zubereitung insbesondere zur Prophylaxe und/oder Therapie von Akne und/oder den mit Akne verbundenen Begleiterkrankungen der Haut verwendet. Hier konnte überraschend festgestellt werden, daß die erfindungsgemäße Zubereitung bei Akne und/oder den mit Akne verbundenen Begleiterkrankungen der Haut sehr schnell zu einer vollständigen Heilung dieser Erkrankungen führte, ohne daß dabei unangenehme oder störende Nebenwirkungen auftraten.

Unter den Begriff Akne im Sinne der vorliegenden Anmeldung fallen alle Erkrankungen der Haut, bei denen eine Ausbildung von Komedonen, Papeln, Pusteln oder Abszessen erfolgt. Insbesondere fallen hier runter Acne cachecticorum, Acne necroticans, Acne varioliformis, Acne picea, Acne vulgaris, Acne conglobata sowie Acne juvenilis.

Die erfindungsgemäße Zubereitung kann zusätzlich noch gängige Therapeutika zur topischen Applikation, wie insbesondere Erythromycin, seine Derivate und/oder entsprechende Salze, Tetracyclin-HCl und/oder Retinolsäure (Tretinoin USP xx 1), enthalten.

Geeignete Mengenverhältnisse der zuvor genannten gängigen Therapeutika sind für

Erythromycin 0,5 - 4 Gew.%,
Tetracyclin-HCl 1 - 5 Gew.%,
Azelainsäure 5 - 20 Gew.% und
Tretinoin 0,025 - 0,1 Gew.%.

Eine weitere Ausführungsform der erfindungsgemäßen Zubereitung sieht vor, daß die Inhaltsstoffe der Zubereitung getrennt verpackt werden. Dies kann insbesondere dadurch geschehen, daß eine erste, trockene Komponente, die das Trägermaterial sowie den Wirkstoff enthält, erst unmittelbar vor Anwendung mit der zweiten Komponente, die aus dem zuvor genannten Lösungsmittel und/oder Wasser besteht, vermischt wird. In der trockenen Komponente ist dann der Wirkstoff sowie das Trägermaterial pulverförmig, granuliert, lyophilisiert oder in sonstiger Weise aufgemacht.

Selbstverständlich besteht desweiteren die Möglichkeit, daß die trockene Komponente dann noch die zuvor genannten Therapeutika enthält.

Vorteilhafte Weiterbildungen der erfindungsgemäßen Zubereitung sind in den Unteransprüchen angegeben.

Die erfindungsgemäße Zubereitung wird nachfolgend anhand von Ausführungsbeispielen näher erläutert.

Für die Herstellung der Zusammensetzungen gemäß der Ausführungsbeispiele 1 - 6 wurden verschiedene Soja-Phospholipide eingesetzt. Hierbei wurde ein Soja-Phospholipid A und ein Soja-Phospholipid B verwendet, wobei das Phospholipid A als Hauptbestandteil 76 ± 3 Gew.% Phosphatidylcholin sowie 3 ± 3 Gew.% Lysophosphatidylcholin und das Phospholipid B als Hauptbestandteil 93 ± 3 Gew.% Phosphatidylcholin sowie 3 ± 3 Gew.% Lyso-Phosphatidylcholin enthielt.

Ausführungsbeispiel 1

In einem Lösungsmittelgemisch, bestehend aus 729 Liter gereinigtem Wasser und 257 Liter Ethanol wurden 330 kg Soja-Phospholipid A, das 100 kg Linolsäure enthielt, aufgenommen. Nach Homogenisierung im Vakuum bei 300 mbar wurde der pH-Wert des entstandenen Gels auf 6,5 ± 1,5 durch Zusatz von Natriumhydroxid eingestellt.

Das entstandene transparente Gel hatte einen Gesamtgehalt an freier und gebundener Linolsäure von 8,05 Gew.%. Die Viskosität des Geles lag bei 5000 ± 3000 mPa.s.

Mikrobiologisch entsprach das Gel den Reinheitsanforderungen der Kategorie 2 des DAB 10 für Fertigarzneimittel.

Bei sachgemäßer Lagerung, d.h. unter Luftausschluß und einer Lagertemperatur bis 25 °C, war das Gel mindestens 24 Monate haltbar.

Ausführungsbeispiel 2

In einem Lösungsmittelgemisch, bestehend aus 12.793 g gereinigtem Wasser und 3200 g Propylenglykol wurden 4 kg Soja-Phospholipid B, das 1,4 kg gebundene Linolsäure enthielt, aufgenommen. Nach Homogenisierung entstand ein gering transparentes weiches Gel mit einem Gesamtgehalt an freier und gebundener Linolsäure von 7 Gew.% und einem pH-Wert von 6,5 ± 1,5, wobei der pH-Wert durch Zusatz von Natriumhydroxid eingestellt worden war. Die Viskosität des Geles betrug 3000 bis 7000 mPa.s.

Ausführungsbeispiel 3

In 400 Liter gereinigtem Wasser wurden 45 kg Soja-Phospholipid A, das 15,7 kg Linolsäure enthielt, dispergiert. Hiernach wurde die Dispersion so lange homogenisiert, bis eine gleichmäßige Dispersion entstand. Nach Abfiltration wurde in Ampullen abgefüllt.

Die hierbei entstehende Dispersion mit einem Gesamtgehalt an freier und gebundener Linolsäure von etwa 3,5 Gew.% wies einen pH-Wert von 6 ± 1 auf, was darin begründet ist, daß bei der Herstellung zur Einstellung des zuvor genannten pH-Wertes eine entsprechende Menge Natriumhydroxid der Dispersion zugesetzt wurde.

Die so hergestellte Dispersion war mehr als 24 Monate stabil.

Ausführungsbeispiel 4

In einem Lösungsmittelgemisch, bestehend aus 401,4 Liter gereinigtem Wasser und 85 kg Ethanol, wurden 50 kg Phospholipid B, das 17,5 kg Linolsäure enthielt, dispergiert. Die hierbei entstehende trübe Dispersion hatte einen pH-Wert von 6 ± 1, wobei der pH-Wert durch Zusatz von Natriumhydroxid eingestellt worden war.

Der Gesamtgehalt an freier und gebundener Linolsäure betrug 3,5 Gew.%.

Ausführungsbeispiel 5

In einem Lösungsmittelgemisch, bestehend aus 401,4 kg gereinigtem Wasser und 85 kg Isopropanol, wurden 50 kg Phospholipid A, das 17,5 kg Linolsäure enthielt, dispergiert. Hierbei entstand eine trübe Dispersion, die einen pH-Wert von 6 ± 1 aufwies, wobei der zuvor genannte pH-Wert durch Zusatz von Natriumhydroxid eingestellt worden war.

Der Gesamtgehalt an freier und gebundener Linolsäure betrug 3,5 Gew.%.

Ausführungsbeispiel 6

In einem Lösungsmittelgemisch, bestehend aus 12.793 g gereinigtem Wasser und 3200 g Propylenglykol wurden 4 kg Soja-Phospholipid B, das 1,4 kg gebundene Linolsäure sowie zuzüglich einen Zusatz von 400 g freie Linolsäure enthielt, aufgenommen. Nach Homogenisierung entstand ein gering transparentes weiches Gel mit einem Gesamtgehalt an freier und gebundener Linolsäure von 9 Gew.% und einem pH-Wert von 6,5 ± 1,5, wobei der pH-Wert durch Zusatz von Natriumhydroxid eingestellt worden war. Die Viskosität des Geles betrug 3000 bis 7000 mPa.s.

Zum Nachweis der Pharmazeutischen Wirksamkeit wurden zwei Präparationen, hergestellt nach Beispiel 3 und 6, an jeweils 13 juvenilen Probanden untersucht. Bis auf ihre unreine Haut, die Pickel, Pusteln und Akne aufwiesen, besaßen die Probanden eine gute Gesundheit.

Die Probanden wurden während der 8-wöchigen Behandlung täglich mit der Präparation behandelt und zwar derartig, daß sie selbst den Inhalt einer Ampulle, die mit 5 ml der Präparation gemäß Ausführungsbeispiel 3 und 6 gefüllt war, auf die linke Gesichtshälfte auftrugen und dort leicht einrieben. Hierbei wurde so viel Präparation aufgetragen, wie sie von der Haut aufgenommen wurde.

Die rechte Gesichtshälfte blieb bei allen Probanden während des 8-wöchigen Behandlungszeitraumes unbehandelt.

Eine andere kosmetische und/oder pharmazeutische Behandlung war während der Versuchsperiode nicht erlaubt.

Es wurde zu Beginn der Behandlung sowie 2 Wochen, 4 Wochen, 6 Wochen und 8 Wochen nach Beginn der Behandlung eine Bewertung des Hautzustandes vorgenommen. Hierzu wurde zur Objektivierung die jeweils behandelten und nicht behandelten Gesichtsflächen mit einer Folie abgedeckt und von einem Prüfarzt auf dieser Folie die Komedonen und Effloreszenten mit einem Stift markiert.

In der nachfolgenden Tabelle sind die jeweiligen Mittelwerte der Anzahl der Komedonen und Effloreszenten angegeben.

Tabelle 1

Ergebnis der Behandlung mit der gemäß Ausführungsbeispiel 3 hergestellten Präparation

Zahl der Komedonen

	WOCHEN				
Gesichtshälfte	0	2	4	6	8
links	18,0	11,2	5,6	4,9	2,2
rechts	18,9	16,8	15,7	16,1	13,8

Zahl der Effloreszenzen

	WOCHEN				
Gesichtshälfte	0	2	4	6	8
links	15,2	7,1	3,2	2,4	1,1
rechts	15,4	14,7	13,5	13,5	13,2

Tabelle 2

Ergebnis der Behandlung mit der gemäß Ausführungsbeispiel 6 hergestellten Präparation

Zahl der Komedonen

	WOCHEN				
Gesichtshälfte	0	2	4	6	8
links	19,0	12,2	5,9	4,2	1,2
rechts	19,9	17,2	16,3	16,8	15,8

Zahl der Effloreszenzen

	WOCHEN				
Gesichtshälfte	0	2	4	6	8
links	15,7	6,3	2,2	1,8	1,0
rechts	17,4	16,7	14,9	15,2	15,3

Bei keinem der behandelten Probanden konnte im Verlauf der Behandlung und 6 Wochen danach eine Unverträglichkeit oder das Auftreten von Hautreizungen festgestellt werden.

Ausführungsbeispiel 7

Es wurde eine Mischung aus 90 Gew.% Phospholipiden (Soja), enthaltend
80 Gew.% 1,2-Diacylglycero-3-phosphocholin,
8 Gew.% 1,2-Diacylglycero-3-phosphat,
4 Gew.% 1,2-Diacylglycero-3-phosphoethanolamin und
8 Gew.% weitere, nicht mehr spezifizierte Phospholipide
mit 90 Gew.% einer physiologischen Kochsalzlösung (1 Gew.% Natriumchlorid in Wasser) hergestellt.
Das Gesamt-Acylrest-Verhältnis der zuvor genannten Phospholipide war wie folgt:
61 - 73 Gew.% Linolsäurerest,
10 - 14 Gew.% Palmitinsäurerest,
8 - 12 Gew.% Ölsäurerest,
4 - 6 Gew.% Linolensäurerest,
3 - 5 Gew.% Stearinsäurerest und
2 Gew.% andere Fettsäurereste.

Die zuvor beschriebene Zubereitung stellte eine liposomale Dispersion dar, die steril produziert wurde und somit keine Konservierungsstoffe enthielt. Diese liposomale Dispersion ist filtrierbar und kann direkt auf die Haut aufgetragen werden.

Ausführungsbeispiel 8

Es wurde ein Gel unter Verwendung des im Ausführungsbeispiel 7 beschriebenen Phospholipids hergestellt, wobei das Gel 20 Gew.% des Phospholipids gemäß Ausführungsbeispiel 7, 16 Gew.% Ethanol und 64 Gew.% Wasser enthielt.

Die so hergestellte liposomale Zusammensetzung, konnte direkt auf die Haut aufgetragen werden.

Bei den nachfolgend aufgeführten Beispielen 9 bis 25 wurde jeweils das im Ausführungsbeispiel 7 beschriebene Phospholipidgemisch verwendet.

Ausführungsbeispiel 9

Es wurde eine Lösung hergestellt, die
2 Gew.% Erythromycin,
16 Gew.% Phospholipid, und
82 Gew.% Propylenglykol
enthielt. Diese Lösung konnte direkt auf die Haut aufgetragen werden.

Ausführungsbeispiel 10

Es wurde eine Lösung hergestellt, die
2 Gew.% Erythromycin,
20 Gew.% Phospholipid,
16 Gew.% Ethanol und
62 Gew.% Propylenglykol
enthielt. Auch diese Lösung konnte direkt auf die Haut aufgetragen werden.

Ausführungsbeispiel 11

Es wurde eine Salbe hergestellt, die
2 Gew.% Erythromycin,
12 Gew.% Phospholipid und
86 Gew.% Cetylstearylalkohol
enthielt.

Ausführungsbeispiel 12

Es wurde eine Salbe hergestellt, die
2,5 Gew.% Erythromycin,
18 Gew.% Phospholipid,
20 Gew.% Propylenglykol und
59,5 Gew.% Cetylstearylalkohol
enthielt.

Ausführungsbeispiel 13

Es wurde ein Gel hergestellt, das
4 Gew.% Erythromycin,
20 Gew.% Phospholipid,
16 Gew.% Ethanol und
60 Gew.% Wasser
enthielt.

Ausführungsbeispiel 14

Es wurde eine liposomale Dispersion hergestellt, wobei die liposomale gebrauchsfertige Dispersion,
1 Gew.% Erythromycin,
10 Gew.% Phospholipid,
16 Gew.% Ethanol und
73 Gew.% Wasser
enthielt.

Zur Herstellung der zuvor genannten Dispersion wurde Erythromycin in einer Flasche vorgelegt und mit dem, das Phospholipid enthaltenden Lösungsmittel (Ethanol, Wasser) durch Schütteln in Lösung gebracht. Die gebrauchsfertige liposomale Dispersion ist erst unmittelbar vor der ersten Anwendung herzustellen und zur baldigen Anwendung bestimmt und dementsprechend kühl zu lagern.

Ausführungsbeispiel 15

Es wurde, wie im Ausführungsbeispiel 14 beschrieben, aus den folgenden Bestandteilen eine gebrauchsfertige liposomale Dispersion hergestellt
1,2 Gew.% Erytromycin,
10 Gew.% Phospholipid,
20 Gew.% Propylenglykol und
68 Gew.% Wasser.

Auch diese liposomale Dispersion ist erst unmittelbar vor Anwendung herzustellen und zum baldigen Gebrauch zu verwenden.

Ausführungsbeispiel 16

Es wurden 2 Systeme jeweils getrennt hergestellt, die erst unmittelbar direkt vor der ersten Anwendung miteinander vermischt werden.

Hierbei enthielt das 1. System
1,2 Gewichtsteile Erythromycin und
20 Gewichtsteile Ethanol,
während das 2. System aus
5 Gewichtsteile Phospholipid,
16 Gewichtsteile Propylenglykol und
57,8 Gewichtsteile Wasser
bestanden.

Die zuvor genannten beiden System wurden erst unmittelbar vor ihrer Anwendung zusammengegeben und vermischt, wobei eine gebrauchsfertige, liposomale Dispersion entstand, die zum baldigen Verbrauch bestimmt war.

Ausführungsbeispiel 17

Es wurde eine Salbe hergestellt, die die folgenden Bestandteile aufwies:
3 Gew.% Tetracyclin-HCl,
17 Gew.% Phospholipid, und
80 Gew.% Vaseline.

Ausführungsbeispiel 18

Es wurde eine Salbe hergestellt, enthaltend
2,5 Gew.% Tetracyclin-HCl,
22,5 Gew.% Phospholipid,
25 Gew.% Wollwachs,
10 Gew.% Propylenglykol und
40 Gew.% Vaseline.

Ausführungsbeispiel 19

Es wurde ein Gel hergestellt, das
5 Gew.% Azelainsäure,
20 Gew.% Phospholipid,
16 Gew.% Propylenglykol und
59 Gew.% Wasser
enthielt.

Ausführungsbeispiel 20

Es wurde ein Gel hergestellt, das
15 Gew.% Azelainsäure,
20 Gew.% Phospholipid,
16 Gew.% Ethanol und
49 Gew.% Wasser
enthielt.

Ausführungsbeispiel 21

Es wurde eine Creme, enthaltend
10 Gew.% Azelainsäure,
20 Gew.% Phospholipid,
16 Gew.% Propylenglykol,
12 Gew.% Mono- und Diglyceride sowie
42 Gew.% Wasser
enthielt.

Ausführungsbeispiel 22

Es wurde ein Gel hergestellt, das
0,025 Gew.% Tretinoin,
20 Gew.% Phospholipid,
16 Gew.% Ethanol und
63,975 Gew.% Wasser
enthielt.

Ausführungsbeispiel 23

Es wurde ein Gel hergestellt, das
0,05 Gew.% Tretinoin,
10 Gew.% Phospholipid,
16,75 Gew.% Ethanol,
2,2 Gew.% Xantan und
71 Gew.% Wasser
enthielt.

Ausführungsbeispiel 24

Es wurde eine Lösung hergestellt, die
0,5 Gew.% Tretinoin,
10 Gew.% Phospholipid,
24 Gew.% Macrogol 400,
17,95 Gew.% Ethanol und
48 Gew.% Propylenglykol
enthielt.

Ausführungsbeispiel 25

Es wurde eine Creme hergestellt, die
0,05 Gew.% Tretinoin,
16 Gew.% Phospholipid,
24 Gew.% Cetystearylalkohol,
22 Gew.% Propylenglykol sowie
37,95 Gew.% Wasser
enthielt.

Zur Überprüfung der Wirksamkeit der vorstehend beschriebenen Zubereitung wurde ein weiterer Probandenversuch durchgeführt, wobei bei diesem dritten Probandenversuch die befallenen Hautbereiche von 14 Probanden mit einer liposomalen Dispersion gemäß Ausführungsbeispiel 7 zweimal täglich behandelt wurden.

Die Auswertung erfolgte durch Markierung der Komedone und der entzündeten Läsionen auf einer Projektionsfolie, wie dies vorstehend beschrieben ist.

Desweiteren wurden durch direkten Kontakt der Haut mit einem Gemisch aus n-Hexan und Isopropanol (V:V; 3:2) Hautoberflächen Lipide entnommen. Die Bestimmung der Gesamtlinolsäure in diesen Proben erfolgte gaschromatographisch nach Überführung in Methylester.

Die Ergebnisse dieses weiteren Probandenversuches sind in Tabelle 3 dargestellt. Die Mittelwerte von 14 Probanden für die Anzahle der Komedonen und der Effloreszenzen sowie des Linolsäuregehaltes der Hautoberfläche sind in Abhängigkeit der Behandlungszeit in der Abbildung 1 dargestellt.

Wie der Abbildung 1 eindeutig zu entnehmen ist, ist bereits nach einer kurzen Behandlungszeit von 2 Wochen die Anzahl der Komedonen und Effloreszenzen im Mittel um etwa 50 %, bezogen auf den Anfangswert, zurückgegangen. Nach einer Behandlungszeit von 8 Wochen mit der Zubereitung gemäß Ausführungsbeispiel 7 tritt eine deutliche weitere Reduzierung der Anzahl der Komedone und Effloreszenzen auf, wobei gleichzeitig der Linolsäuregehalt im extrahierten Zustand Hautoberflächenbereich steigt.

In Abbildung 2 ist im Vergleich die Wirksamkeit verschiedener bekannter Aknemittel dargestellt, wobei die dort in Form eines Blockdiagramms wiedergegebenen Ergebnisse die prozentuale Absenkung nach einer Behandlungszeit von 2 Monaten mit dem jeweiligen Mittel darlegen.

Aus dem Vergleich der Abbildungen 1 und 2 ergibt sich eindeutig, daß die Behandlung mit der liposomalen Dispersion gemäß Ausführungsbeispiel 7 wesentlich bessere Ergebnisse ergibt als das bisher bekannte und am meisten wirksame Akne-Mittel Isotretinoin. Desweiteren ist festzuhalten, daß bei keinem der durchgeführten und vorstehend beschriebenen Probandenversuche Nebenwirkungen auftraten, was bei einer Behandlung mit Isotretinoin nicht der Fall ist.

Ausführungsbeispiel 26

Es wurde eine Zusammensetzung unter Verwendung des eingangs genannten Soja-Phospholipids A hergestellt, wobei die Zusammensetzung eine erste, im Behälter 1 verpackte Komponente sowie eine zweite, im Behälter 2 verpackte Komponente aufwies.

Der Behälter 1 wies die folgenden Inhaltsstoffe auf:

demineralisiertes Wasser	73,83 g
Ethanol DAB 9	10 g
10 %ige NaOH	0,17 g

Der Behälter 2 beinhaltete folgende Inhaltsstoffe:

Phospholipid A	10 g
Ethanol DAB 9	6 g

Ausführungsbeispiel 27

Es wurde ebenfalls eine auf zwei Behälter aufgeteilte Zusammensetzung hergestellt. Hierbei wies der Behälter 1

demineralisiertes Wasser	73,83 g
Isopropylalkohol	10 g
Natriumhydroxid, 10 %ig	0,17 g

und
der Behälter 2

Phospholipid A	10 g
Isopropylalkohol	6 g

auf.

Ausführungsbeispiel 28

Es wurde eine weitere Zusammensetzung hergestellt, wobei im Behälter 1

physiologische Kochsalzlösung (1 %ig)	73,83 g
Ethanol DAB 9	10 g
Natriumhydroxid 10 %ig	0,17 g

waren und sich im Behälter 2

Phospholipid A	10 g
Ethanol DAB 9	6 g

befanden.

Ausführungsbeispiel 29

Wie bei den vorstehend genannten Ausführungsbeispielen 26 bis 28 wurde eine aus zwei Komponenten bestehende Zusammensetzung erstellt, wobei der erste Behälter 1

physiologische Kochsalzlösung (1 %ig)	73,83 g
Isopropylalkohol	10 g
Natriumhydroxidlösung (10 %ig)	0,17 g

und der zweite Behälter

Phospholipid A	10 g sowie
Isopropylalkohol	6 g

aufwies.

Die zuvor in den Ausführungsbeispielen 26 bis 29 in separate Behältern abgefüllten Bestandteile der Zusammensetzung wurden durch kurzes Schütteln (30 Sekunden) miteinander vermischt. Hierbei entstand aus den Bestandteilen des Ausführungsbeispiels 26 ein bräunliches, liposomales Gel mit einem mittleren Liposomendurchmesser von 380 nm und einen pH-Wert von 6,8, aus den Bestandteilen des Ausführungsbeispiel 27 ebenfalls ein bräunliches, liposomales Gel mit einer mittleren Teilchengröße von 311 nm und einem pH-Wert von 7,0, aus den Bestandteilen des Ausführungsbeispiels 28 eine milchige, liposomale Flüssigkeit mit einer mittleren Teilchengröße von 540 nm und einem pH-Wert von 6,6 und aus den Bestandteilen des Ausführungsbeispiels 29 eine gelblich flüssige liposomale Zubereitung mit einer mittleren Liposomenteilchengröße von 281 nm und einem pH-Wert von 6,8.

Tab. 3

Prob.Nr.	Ausgang			nach 2 W.			nach 4 W.			nach 8 W.		
	K.	Ef.	L.S.	K.	Ef.	L.S.	K.	Ef.	L.S.	K.	Ef.	L.S.
1	28	19	0,64	23	8	1,62	16	7	3,49	12	6	3,57
2	32	4	0,34	15	2	1,45	11	2	1,94	11	0	2,97
3	6	10	0,40	4	4	4,45	1	7	1,21	0	5	2,90
4	38	27	0,57	23	6	0,54	7	13	3,33	7	0	3,24
5	16	15	1,40	16	3	1,90	18	0	2,63	14	0	3,47
6	0	33	1,30	0	19	2,13	0	11	2,80	0	3	3,40
7	35	25	1,94	22	7	3,22	19	1	6,28	17	0	4,59
8	0	12	0,86	0	8	4,04	0	9	4,00	0	6	3,82
9	34	4	2,07	10	2	2,93	12	0	3,49	12	0	3,28
10	34	15	1,12	16	3	3,37	13	2	4,83	6	0	4,62
11	30	29	1,70	17	25	2,27	18	23	2,25	13	8	3,10
12	28	15	1,77	16	2	1,96	11	3	2,12	4	4	3,24
13	2	9	1,70	0	4	2,03	0	7	2,40	3	5	2,92
14	9	8	0,57	6	0	1,20	4	0	1,79	0	0	3,14

K = Komedone
Ef. = Effloreszenz
L.S. = % Linolsäure im Eluat bezogen auf den Gesamtfettsäuregehalt

Patentansprüche

1. Pharmazeutische und/oder kosmetische Zubereitung zur topischen Anwendung, wobei die Zubereitung neben mindestens einem Wirkstoff desweiteren ein Trägermaterial für den Transport des Wirkstoffes in die Haut aufweist, dadurch gekennzeichnet, daß die Zubereitung als Wirkstoff Linolsäure und/oder mindestens ein Linolsäurederivat enthält.

2. Zubereitung nach Anspruch 1, dadurch gekennzeichnet, daß die Zubereitung als Trägermaterial Liposome aufweist.

3. Zubereitung nach Anspruch 2, dadurch gekennzeichnet, daß die Liposome von mindestens einem Phospholipid gebildet sind.

4. Zubereitung nach Anspruch 3, dadurch gekennzeichnet, daß das Phospholipid ein Gemisch von Phospholipiden darstellt.

5. Zubereitung nach Anspruch 3 oder 4, dadurch gekennzeichnet, daß das Phospholipid ein pflanzliches Phospholipid, insbesondere ein Sonnenblumen- oder Soja-Phospholipid, ist.

6. Zubereitung nach einem der vorangehenden Ansprüche, dadurch gekennzeichnet, daß die Zubereitung 5 Gew.% bis 50 Gew.%, insbesondere 15 Gew.% bis 30 Gew.% des Trägermaterials und vorzugsweise eines phospholipidischen Trägermaterials, bezogen auf die anwendungsfertige Zubereitung, enthält.

7. Zubereitung nach Anspruch 5 oder 6, dadurch gekennzeichnet, daß das pflanzliche Phospholipid zwischen 70 Gew.% und 100 Gew.% 1,2-Diacylglycero-3-Phosphocholin aufweist.

8. Zubereitung nach einem der vorangehenden Ansprüche, dadurch gekennzeichnet, daß die Zubereitung einen wasserlöslichen Alkohol und/oder Wasser enthält.

9. Zubereitung nach einem der vorangehenden Ansprüche, dadurch gekennzeichnet, daß die Zubereitung Linolsäure und/oder mindestens ein Linolsäurederivat aufweist, die bzw. das chemisch und/oder physikalisch an das Trägermaterial gebunden ist.

10. Zubereitung nach Anspruch 9, dadurch gekennzeichnet, daß die Zubereitung Linolsäure und/oder das mindestens eine Linolsäurederivat aufweist, die bzw. das chemisch an das Trägermaterial gebunden ist.

11. Zubereitung nach einem der vorangehenden Ansprüche, dadurch gekennzeichnet, daß die Zubereitung neben der Linolsäure und/oder dem mindestens einen Linolsäurederivat desweiteren noch ein mit Linolsäure und/oder dem Linolsäurederivat acyliertes Phospholipid aufweist.

12. Zubereitung nach Anspruch 10, dadurch gekennzeichnet, daß die Zubereitung als Wirkstoff ein mit Linolsäure bzw. dem Linolsäurederivat acyliertes Phospholipid enthält.

13. Zubereitung nach Anspruch 11 oder 12, dadurch gekennzeichnet, daß das mit Linolsäure bzw. dem Linolsäurederivat acylierte Phospholipid ein 1,2-Diacylglycero-3-Phosphocholin ist.

14. Zubereitung nach einem der Ansprüche 10 bis 13, dadurch gekennzeichnet, daß die Zubereitung ein mit Linolsäure oder einem Linolsäurederivat acyliertes Phospholipid enthält, wobei mindestens 60 Gew.% des Acylrestes Linolsäure und/oder das Linolsäurederivat sind.

15. Zubereitung nach Anspruch 14, dadurch gekennzeichnet, daß die Zubereitung ein mit Linolsäure oder einem Linolsäurederivat acyliertes Phospholipidgemisch enthält.

16. Zubereitung nach Anspruch 15, dadurch gekennzeichnet, daß die Zubereitung ein solches acyliertes Phospholipidgemisch enthält, bei dem die in dem Gemisch enthaltenen Acylreste zu
    61 - 73 Gew.% aus dem Linolsäurerest,
    10 - 14 Gew.% aus dem Palmitinsäurerest,
    8 - 12 Gew.% aus dem Ölsäurerest,
    4 - 6 Gew.% aus dem Linolensäurerest,
    3 - 5 Gew.% aus dem Stearinsäurerest und/oder
    2 Gew.% aus anderen Fettsäureresten
    bestehen.

17. Zubereitung nach Anspruch 15 oder 16, dadurch gekennzeichnet, daß das Phospholipidgemisch eines oder mehrerer anderer 1,2-Diacylglycero-3-phosphate, ausgewählt aus der Gruppe, bestehend aus
    1,2-Diacylglycero-3-phosphoethanolamin,
    1,2-Diacylglycero-3-phosphoinositol,
    1,2-Diacylglycero-3-phosphoserin,
    1,2-Diacylglycero-3-phosphoglycerol und
    1,2-Diacylglycero-3-phosphat
    enthält.

18. Zubereitung nach einem der vorangehenden Ansprüche, dadurch gekennzeichnet, daß in dem 1,2-Diacylglycero-3-phosphocholin der 1-Acylrest
    45 - 61 Gew.% Linolsäurereste,
    19 - 26 Gew.% Palmitinsäurereste,
    8 - 12 Gew.% Ölsäurereste,
    4 - 6 Gew.% Linolensäurereste,
    6 - 9 Gew.% Stearinsäurereste und/oder
    2 Gew.% andere Fettsäurereste
    enthält.

19. Zubereitung nach einem der vorangehenden Ansprüche, dadurch gekennzeichnet, daß in dem 1,2-Diacylglycero-3-phosphocholin der 2-Acylrest
    77 - 85 Gew.% Linolsäurereste,
    1 - 2 Gew.% Palmitinsäurereste,
    8 - 12 Gew.% Ölsäurereste,
    4 - 6 Gew.% Linolensäurereste,
    0 - 1 Gew.% Stearinsäurereste und/oder
    2 Gew.% andere Fettsäurereste
    enthält.

20. Zubereitung nach einem der vorangehenden Ansprüche, dadurch gekennzeichnet, daß die Zubereitung zwischen 1 Gew.% und 30 Gew.%, vorzugsweise zwischen 3 Gew.% und 18 Gew.%, Linolsäure und/oder Linolsäurederivat aufweist.

21. Zubereitung nach einem der vorangehenden Ansprüche, dadurch gekennzeichnet, daß die Zubereitung ein Gel ist und zwischen
    30 Gew.% und 93 Gew.% Wasser,
    0 Gew.% und 20 Gew.% Lösungsmittel,
    1 Gew.% und 30 Gew.% Linolsäure und/oder Linolsäurederivat und
    5 Gew.% und 50 Gew.% Phospholipid
    aufweist.

22. Zubereitung nach einem der Ansprüche 1 bis 20, dadurch gekennzeichnet, daß die Zubereitung ein flüssiges System ist und zwischen
    69 Gew.% und 94 Gew.% Wasser,
    0 Gew.% und 20 Gew.% Lösungsmittel,
    1 Gew.% und 30 Gew.% Linolsäure und/oder Linolsäurederivat und
    5 Gew.% und 30 Gew.% Phospholipid
    enthält.

23. Zubereitung nach Anspruch 22, dadurch gekennzeichnet, daß die Zubereitung als Lösungsmittel Wasser aufweist und in gasdichten Ampullen abgepackt ist.

24. Zubereitung nach einem der vorangehenden Ansprüche, dadurch gekennzeichnet, daß die Zubereitung eine erste Komponente und eine hiervon getrennt verpackte zweite Komponente umfaßt, wobei die erste Komponente
    ein Lösungsmittel,
    mindestens einen Elektrolyten und/oder
    Wasser,
    und die zweite Komponente
    das Trägermaterial,
    die Linolsäure und/oder das Linolsäurederivat sowie
    ggf. Lösungsmittel
    enthält.

25. Zubereitung nach Anspruch 24, dadurch gekennzeichnet, daß eine der beiden Komponenten, vorzugsweise die erste Komponente, noch einen pH-Regulator umfaßt.

26. Zubereitung nach Anspruch 24 oder 25, dadurch gekennzeichnet, daß die zweite Komponente aus Linolsäure und/oder dem mindestens einen Linolsäurederivat besteht, wobei die Linolsäure bzw. das Linolsäurederivat chemisch und/oder physikalisch an das phospholipidische Trägermaterial gebunden ist.

27. Zubereitung nach einem der Ansprüche 24 bis 26, dadurch gekennzeichnet, daß die erste und/oder die zweite Komponente noch übliche Hilfsstoffe aufweisen.

28. Zubereitung nach einem der Ansprüche 24 bis 27, dadurch gekennzeichnet, daß die erste Komponente
50 Gew.% bis 80 Gew.% Wasser sowie
0 Gew.% bis 20 Gew.% Lösungsmittel,
und die zweite Komponente
3 Gew.% bis 15 Gew.% des mit Linolsäure oder dem mindestens einen Linolsäurederivat acylierten phospholipidischen Trägermaterials sowie
0 Gew.% bis 15 Gew.% Lösungsmittel enthält.

29. Zubereitung nach Anspruch 28, dadurch gekennzeichnet, daß das Wasser in der ersten Komponente eine wäßrige physiologische Kochsalzlösung ist.

30. Zubereitung nach einem der vorangehenden Ansprüche, dadurch gekennzeichnet, daß die Zubereitung einen weiteren Wirkstoff, ausgewählt aus der Gruppe Erythromycin, ein Erythromycinsalz, ein Erythromycinderivat, Tetracyclin, Azelainsäure und/oder Retinolsäure enthält.

31. Verwendung der Zubereitung nach einem der vorangehenden Ansprüche zur Prophylaxe und/oder Therapie von Akne und/oder der mit Akne verbundenen Begleiterkrankungen der Haut.

32. Verwendung der Zubereitung nach einem der Ansprüche 1 bis 29 zur Prophylaxe und/oder Therapie von unreiner Haut.

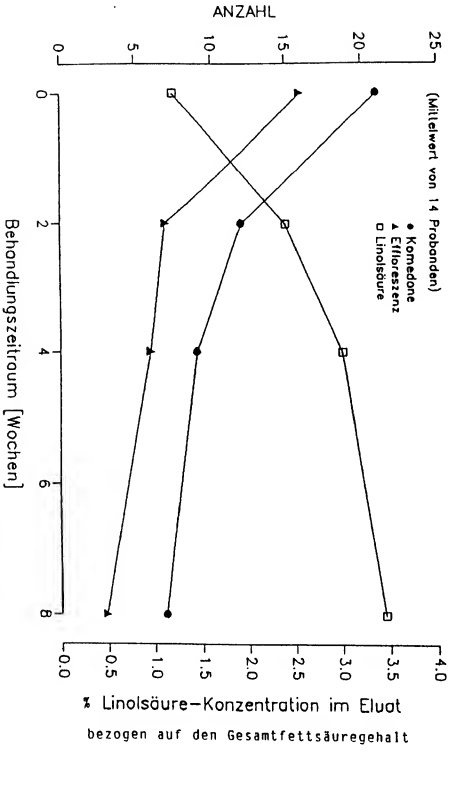

ANZAHL
25
20
15
10
5
0
(Mittelwert von 14 Probanden)
● Komedone
▲ Efflureszenz
□ Linolsäure
0
2
4
6
8
Behandlungszeitraum [Wochen]
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
% Linolsäure-Konzentration im Eluat
bezogen auf den Gesamtfettsäuregehalt

Abb. 1

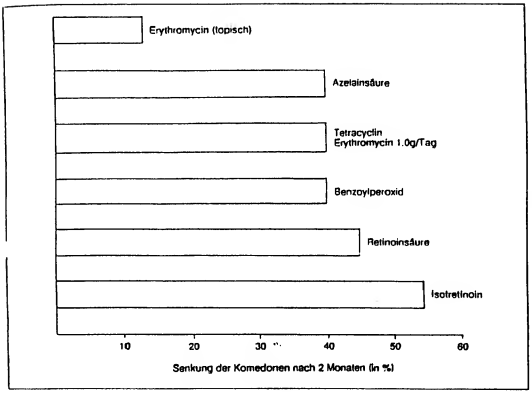

Erythromycin (topisch)
Azelainsäure
Tetracyclin
Erythromycin 1.0g/Tag
Benzoylperoxid
Retinoinsäure
Isotretinoin
10
20
30
40
50
60
Senkung der Komedonen nach 2 Monaten (in %)

Abb. 2

Europäisches Patentamt

EUROPÄISCHER TEILRECHERCHENBERICHT

der nach Regel 45 des Europäischen Patentübereinkommens für das weitere Verfahren als europäischer Recherchenbericht gilt

Nummer der Anmeldung: EP 93 11 2323

EINSCHLÄGIGE DOKUMENTE

Kategorie	Kennzeichnung des Dokuments mit Angabe, soweit erforderlich der maßgeblichen Teile	Betrifft Anspruch	KLASSIFIKATION DER ANMELDUNG (Int.Cl.5)
X	SEIFEN- öLE-FETTE-WACHSE Bd. 117, Nr. 10 , 26. Juni 1991 , AUGSBURG (DE) Seiten 372 - 378 XP228115 J. RÖDING ET AL. 'beeinflussung der hautfeuchtigkeit durch liposomen, stabilisierung von pflegenden ölen und lipophilen wirkstoffen mit liposomen' * Seite 376 - Seite 377, Absatz 3.2 * ---	1-15,20	A61K9/127 A61K31/20 A61K31/685 A61K7/00
X	SEIFEN-öLE-FETTE -WACHSE Bd. 114, Nr. 14 , 1. September 1988 , AUGSBURG (DE) Seiten 531 - 534 XP24821 H. LAUTENSCHLÄGER ET AL. 'über die verwendung von liposomen aus soja-phospholipiden in der kosmetik' * Seite 531 - Seite 532, Absatz 1 2 * ---	1-10	
X	EP-A-0 331 489 (PARKE DAVIS PTY. LTD.) * das ganze Dokument * --- -/--	1,8,20, 30-32	RECHERCHIERTE SACHGEBIETE (Int.Cl.5) A61K

UNVOLLSTÄNDIGE RECHERCHE

Nach Auffassung der Recherchenabteilung entspricht die vorliegende europäische Patentanmeldung den Vorschriften des Europäischen patentübereinkommens so wenig, daß es nicht möglich ist, auf der Grundlage einiger Patentansprüche sinnvolle Ermittlungen über den Stand der Technik durchzuführen.

Vollständig recherchierte Patentansprüche:

Unvollständig recherchierte Patentansprüche:

Nicht recherchierte Patentansprüche:

Grund für die Beschränkung der Recherche:

Siehe Ergänzungsblatt C

Recherchenort	Abschlußdatum der Recherche	Prüfer
DEN HAAG	19. November 1993	BENZ, K

KATEGORIE DER GENANNTEN DOKUMENTEN

X : von besonderer Bedeutung allein betrachtet
Y : von besonderer Bedeutung in Verbindung mit einer anderen Veröffentlichung derselben Kategorie
A : technologischer Hintergrund
O : nichtschriftliche Offenbarung
P : Zwischenliteratur

T : der Erfindung zugrunde liegende Theorien oder Grundsätze
E : älteres Patentdokument, das jedoch erst am oder nach dem Anmeldedatum veröffentlicht worden ist
D : in der Anmeldung angeführtes Dokument
L : aus anderen Gründen angeführtes Dokument

& : Mitglied der gleichen Patentfamilie, übereinstimmendes Dokument

EPO FORM 1503 03.82 (P04C09)

Europäisches Patentamt

EUROPÄISCHER TEILRECHERCHENBERICHT

Nummer der Anmeldung
EP 93 11 2323

EINSCHLÄGIGE DOKUMENTE

Kategorie	Kennzeichnung des Dokuments mit Angabe, soweit erforderlich der maßgeblichen Teile	Betrifft Anspruch	KLASSIFIKATION DER ANMELDUNG (Int.Cl.5)
X	DE-A-40 21 082 (LAUTENSCHLÄGER)	1-15, 20-22	
Y	* das ganze Dokument *	16-19, 23-29	
	* Seite 3, Zeile 32 - Zeile 33 *		
	* Seite 4, Zeile 8 - Zeile 10 *		
	* Seite 5, Zeile 13 *		
	* Seite 5, Zeile 33 *		
	* Seite 8; Beispiel 3 *		

Y	EP-A-0 147 741 (A. NATTERMANN & CIE. GMBH)	16-19	
	* Ansprüche 1-7 *		

A	FR-A-2 649 322 (LABORATOIRES NATURA MEDICA)	1-32	RECHERCHIERTE SACHGEBIETE (Int.Cl.5)
	* Seite 1, Zeile 1 - Seite 5, Zeile 28 *		
	* Seite 7; Beispiel 4 *		

Y	US-A-4 830 858 (PAYNE ET AL.)	23-29	
	* das ganze Dokument *		

BEMERKUNG: Obwohl die Ansprüche 31,32 sich auf ein Verfahren zur Behandlung des menschlichen/ tierischen Körpers beziehen, wurde die Recherche durchgeführt und gründet sich auf die angeführten Wirkungen der Zusammensetzung

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(54) **Bioactive compounds associated with liposomes and their use in pharmaceutical preparations.**

(57) A method of treating a patient with bioactive agents comprises providing aggregates of bioactive agents in association with liposomes, wherein the aggregates comprise liposomes having outer surfaces, and bioactive agents, wherein the bioactive agent is associated with the outer surfaces of the liposomes such that biological activity is not substantially diminished when the aggregates are administered in a pharmaceutically effective amount to a patient, and administering the aggregates to the patient. Preferred bioactive agents are TGF-β and M-CSF. A preferred method comprises aggregates of negatively-charged liposomes and TGF-β, having a net positive charge. In addition, a composition of matter comprises aggregates of TGF-β in association with liposomes. A pharmaceutical composition comprises the aggregates of TGF-β in association with liposomes in conjunction with pharmaceutically-acceptable carriers. A preferred method comprises aggregates of positively-charged liposomes and M-CSF, having a net negative charge. In addition, a composition of matter comprises aggregates of M-CSF in association with liposomes. A pharmaceutical composition comprises the aggregates of M-CSF in association with liposomes in conjunction with pharmaceutically-acceptable carriers.

EP 0 393 707 A2

Bioactive Compounds Associated with Liposomes and their use in Pharmaceutical Preparations

FIELD OF THE INVENTION

The present invention relates to a method of treating a patient with bioactive compounds such as transforming growth factor, type β, hereinafter "TGF-β" and colony-stimulating factors, such as macrophage colony-stimulating factor, hereinafter "M-CSF." In addition, the present invention relates to compositions of bioactive compounds associated with liposomes such as TGF-β associated with liposomes and M-CSF associated with liposomes. More particularly, the invention relates to pharmaceutical compounds comprising a bioactive compound associated with ionically-charged liposomes in conjunction with a pharmaceutically-acceptable carrier for administration to patients. Such pharmaceutical compounds may be TGF-β associated with liposomes or M-CSF associated with liposomes.

BACKGROUND OF THE INVENTION

Bioactive compounds such as TGF-β and M-CSF may be administered for the treatment of a variety of diseases. The administration of such bioactive compounds, however, may be hampered by limited bioavailability. Once a biological agent is absorbed or injected into the blood stream, the rate, extent, and pattern of the initial distribution are determined, inter alia, by the physicochemical characteristics of the drug. Compounds that exhibit a charge at physiologic ph may reach the target area in only diminished quantities. For example, the positive charge of TGF-β may cause the peptide to adhere to cellular membranes, such as vascular membranes, or to plasma proteins, such as albumin, or other components of blood such that the half-life of the agent is decreased and biological activity is reduced. Similarly, the negative charge of M-CSF may hamper the ability of that agent to reach the target site.

Any reduction in bioavailability is exacerbated by administration through the blood system because compounds in the blood stream are vulnerable to the body's clearing mechanisms. Recently, liposomes have been used to protect various biologically active agents from such host clearing mechanisms by entrapment of the biological agent within the liposome.

Entrapment of biologically active agents within liposomes, however, requires a relatively large amount of agent. This is problematic for peptides, like TGF-β, that are produced at great expense and in very small quantities. For example, an improved method for the production of TGF-β, described as presenting a five-fold increase in recovery over previous isolation techniques, yields only 2.5 μg per unit of human platelets. Cone et al. "An Improved Method of Purification of Transforming Growth Factor, Type Beta from Platelets," Analytical Biochemistry 168: 71-74 (1988). Because relatively large quantities of biologically active agent are dispersed in an aqueous layer in the preparation of a liposome-entrapped biological agent, this method is not suitable for use with peptides that are not readily available in large quantities, such as TGF-β.

In addition, liposome entrapment of biological agents may be inferior to liposome surface association with biological agents, such as TGF-β and M-CSF, that act by first attaching to receptors on the membranes of cells because agents entrapped within the aqueous layer of a liposome will not have access to cellular receptors. Recent studies have shown that TGF-β and M-CSF do attach to cellular receptors found on a wide variety of different cell types. Wakefield, L.M. et. al., "Distribution and Modulation of the Cellular Receptor for Transforming Growth Factor-Beta," J. Cell. Bio. 105: 965-975 (1987). Stanley, E.R. et al., "Regulation of macrophage production by colony-stimulating factor", In Mononuclear Phagocytes - Functional Aspects, Part 1, ed. R. van Furth (1980); Byrene, P.V., et al., "This Distribution of Cells Having Receptors for a Colony-Stimulating Factor (CSF-1) in Murine Tissues," J. Cell. Biol. 91:848 (1981). Thus, entrapment of TGF-β and M-CSF in liposomes may diminish biological activity of the bioactive agents.

Accordingly, there is a need in the art for a method of administering bioactive agents such as TGF-β and M-CSF to patients that will maximize the quantity of biological agent that reaches the target site, protect the bioactive agents from the body's clearing mechanisms, and maintain the biological activity of the bioactive agents, and that is suitable in use with a peptide that is obtainable in small amounts. There is also a need in the art for compounds and compositions for use in the method.

SUMMARY OF THE INVENTION

The present invention overcomes the problems and disadvantages of the prior art by providing a method of treating a patient with bioactive agents, comprising providing aggregates of bioactive agents in association with liposomes, wherein the aggregates comprise liposomes having outer surfaces and bioactive agents, wherein the bioactive agent is associated with the outer surfaces of the liposomes such that biological activity is not substantially diminished when the aggregates are administered in a pharmaceutically effective amount to the patient, and administering the aggregates to the patient. The present invention also provides a method of treating patients with TGF-β, comprising providing aggregates of TGF-β in association with liposomes, wherein the aggregates comprise liposomes having outer surfaces, and TGF-β, wherein the TGF-β is associated with the outer surfaces of the liposomes such that biological activity is not substantially diminished when the aggregates are administered in a pharmaceutically effective amount to the patient, and administering the aggregates to the patient. The invention also provides a preferred embodiment of the method of treating patients with TGF-β wherein negatively-charged liposomes are used to form the aggregates of TGF-β in association with liposomes. In addition, the present invention provides a composition of matter, comprising aggregates of TGF-β in association with liposomes. The invention also provides a pharmaceutical composition comprising pharmaceutically effective amounts of the aggregates of TGF-β in association with liposomes in conjunction with pharmaceutically-acceptable carriers.

The present invention also provides a method of treating patients with M-CSF, comprising providing aggregates of M-CSF in association with liposomes, wherein the aggregates comprise liposomes having outer surfaces, and M-CSF, wherein the M-CSF is associated with the outer surfaces of the liposomes such that biological activity is not substantially diminished when the aggregates are administered in a pharmaceutically effective amount to the patient, and administering the aggregates to the patient. The invention also provides a preferred embodiment of the method of treating patients with M-CSF wherein positively-charged liposomes are used to form the aggregates of M-CSF in association with liposomes. In addition, the present invention provides a composition of matter, comprising aggregates of M-CSF in association with liposomes. The invention also provides a pharmaceutical composition comprising pharmaceutically effective amounts of the aggregates of M-CSF in association with liposomes in conjunction with pharmaceutically-acceptable carriers.

Additional objects and advantages of the invention will be set forth in part in the description which follows, and in part may be learned from the description or by practice of the invention. The objects and advantages of the invention will be realized and attained by means of the instrumentalities and combinations particularly pointed out in the appended claims.

It is to be understood that the general description above and the following detailed description are exemplary and explanatory only and do not limit the invention, as claimed.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several exemplary embodiments of the invention and, together with the description, serve to explain the principles of the invention.

Fig. 1 is a graph which depicts the effect of varying lipid/protein ratios on the association of TGF-β with negatively-charged liposomes.

Fig. 2 is a graph which indicates the extent of TGF-β association with neutral liposomes.

Fig. 3 is a graph which indicates the extent of TGF-β association with negatively-charged liposomes.

Fig. 4 is a graph which depicts the effects of TGF-β associated liposomes on delayed type hypersensitivity responses to Listeria.

Fig. 5 is a graph which depicts the effects of TGF-β associated liposomes on delayed type hypersensitivity responses to Listeria.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Reference will now be made in detail to the presently preferred embodiments of the invention, examples of which are illustrated in the accompanying drawings.

As used herein, bioactive agents are peptides, polypeptides, proteins, and glycoproteins that cause a cellular response when administered to patients. The cellular responses observed with bioactive agents range from proliferative effects, nonproliferative effects, differentiation, and other effects such as the suppression of immunoglobulin secretion and adrenal steroidogenesis. The bioactive agents particularly preferred are TGF-β and M-CSF, but this invention is, in no way, limited to the use of these specific agents.

As used herein, TGF-β is a hormonally active polypeptide known as transforming growth factor, type beta. Much progress has been made in identifying and structurally characterizing TGF-β since its discovery only a few years ago. TGF-β consists of two identical polypeptide chains (Mr 12,500), linked by disulfide bonds. The activity of the native molecule is destroyed upon reduction of the disulfide groups.

TGF-β is a ubiquitous molecule that is present in many normal tissues, as well as in transformed cells. Blood platelets, in particular, store relatively large amounts of TGF-β (about 1500 molecules of TGF-β per cell), which supports the view that TGF-β has a role in wound healing.

TGF-β, however, has been found to be a multifunctional growth factor. For example, it has proliferative effects on fibroblasts and osteoblasts and anti-proliferative effects on fibroblasts, epithelial cells, T-lymphocytes, and osteoblasts. In addition, TGF-β has effects that are unrelated to proliferation: it has been shown to suppress immunoglobulin secretion and adrenal steroidogenesis. Sporn and Roberts, "Peptide Growth Factors are Multifunctional," Nature 332: 217-219 (March 17, 1988). It has been suggested that the nature of growth factor action may depend on the presence of other substances, i.e., TGF-β stimulates fibroblasts in the presence of platelet-derived growth factor but inhibits their growth in the presence of epidermal growth factor.

In addition, TGF-β is believed to be a potent regulator of the expression of the processes of adipogenesis, myogenesis, osteogenesis, and epithelial differentiation. Scientists have proposed that the biochemical basis of these multiple cellular actions is due, at least in part, to the ability of TGF-β to alter the architecture of extracellular matrices. See "The Transforming Growth Factors" by Joan Massague and "Negative Regulators of Cell Growth" by John L. Wang and Yen-Ming Hsu, in Oncogenes and Growth Factors, ed. Ralph A. Bradshaw and Steve Prentis (1987).

One function of TGF-β that is of particular interest in this invention is its ability to manipulate the immune system. Recent studies show that TGF-β is at least 10^4 times more potent on a molar basis than the peptide cyclosporin A as a suppressor of T-lymphocytes. Sporn and Roberts, supra. This inhibitory effect makes it potentially useful in the treatment of conditions such as rheumatoid arthritis, systemic lupus erythematosis, autoimmune diabetes, autoimmune throiditis, and multiple sclerosis and other autoimmune diseases. In addition, TGF-β may facilitate allograft transplantation and inhibit certain tumor cells.

Because TGF-β has a pI of 9.5, Seyedin et al., "Cartilage-inducing Factor-B is a Unique Protein Structurally and Functionally related to Transforming Growth Factor-β," J. of Biol. Chem. 262 (5): 1946-49 (1987), at physiologic pH, it has been shown that TGF-β has a net positive charge. Net positive charge, as used herein, refers to the positive ionization state of the polypeptide. Because polypeptides are composed of amino acids that have a certain charge at a particular pH, the ionization states of the individual components yield a net positive or negative charge for the polypeptide. The amino acid composition of TGF-β is such that it bears a net positive charge.

As used herein, the term "M-CSF" relates to a particular type of colony-stimulating factor. Colony-stimulating factors are glycoproteins that regulate the production of hematopoietic cells. At least four distinct colony-stimulating factors control proliferation and differentiation of granulocytes and/or macrophages from hematopoietic precursors.

One particularly preferred agent is the macrophage colony-stimulating factor (M-CSF, also known as CSF-1) that can selectively stimulate the survival, proliferation, and differentiation of mononuclear phagocyte lineage cells. Studies have also shown that M-CSF also stimulates effector functions of mature monocytes such as antifungal activity and lymphokine-induced tumoricidal activity. Stanley, E.R. and Gulbert, L.T., "Method for the Purification, Assay, Characterization and Target Cell Binding of Colony-Stimulating Factor [CSF-1]", J. Immunologic Methods 42: 253-284 (1981); Karbassi, A., et al., "Enhanced Killing of Candida Albicans by Murine Macrophages Treated with Macrophage Colony-Stimulating Factor: Evidence for Augmented Expression of Mannose Receptors", J. Immunology 139: 417-421 (1987); Metcalf, D. "The Molecular Biology and Functions of the Granulocyte macrophage Colony-Stimulating Factors," Blood 67:257 (1986); and Ralph P. and Nakoinz, I., "Stimulation of Macrophage Tumoricidal Activity by Growth and Differentiation Factor CSF-1", Cellular Immunology 105: 270-279 (1987).

The instant application encompasses all forms of M-CSF, including whole intact M-CSF as well as M-CSF that has been truncated or otherwise altered by standard biochemical or recombinant techniques. See, U.S. Application Serial No. 07/304,692, filed February 1, 1989, entitled "Human Colony-Stimulating Factors," to Takahashi, M. et. al.

Studies have shown that, at physiological pH, human M-CSF exhibits a pI of 4.8 and a net negative charge. Similarly, a truncated form of human M-CSF has been shown to exhibit a PI of 4.5. Such recombinant techniques are provided in the copending U.S. Application Serial No. 07/304,692, filed February 1, 1989, entitled "Human Colony-Stimulating Factors," to Takahashi, M. et. al. Net negative charge, as used herein, refers to the negative ionization state of the agent. As discussed above with regard to net positive charge, the charge of an entity is based on the amino acids that comprise the entity. The amino acids that make up at least a part of a glycoprotein confers on the glycoprotein a particular charge.

As used herein, the term "aggregate" means a collection of units or particles into a body, mass, or other grouping. The present invention is not limited by the number of units that form the aggregate of the present invention.

The term "association," as used herein, means any kind of relationship between the parts of the association. The parts may be associated by proximity so that there is no actual contact between the parts. Additionally, the parts may be associated by superficial contact, such as surface-to-surface contact. The association may result from even closer contact such that a member of the association is embedded, partially or fully, in another member of the association.

As used herein, the term "liposome" is to be given a broad meaning. Liposomes, also called lipid vesicles, are aqueous compartments enclosed by a lipid bilayer which range in shape from spherical to elongated.

Liposomes are formed by suspending a suitable lipid in an aqueous medium. This mixture is then shaken to yield a dispersion of liposomes that are quite uniform in size. Alternatively, liposomes can be made by rapidly mixing a solution of lipid in ethanol with water. This can be accomplished by injection of the lipid through a fine needle. The method chosen to prepare a liposome in part determines the form of liposome prepared. The present invention contemplates use of all forms of liposomes, including large unilamellar vesicles, small unilamellar vesicles, and multilamellar vesicles.

Multilamellar vesicles, also called oligolamellar vesicles, can be prepared in any conventional manner, including that employing a rotary vacuum evaporator. Sunamato, J. et al., "A Newly Developed Immunoliposome - an Egg Phosphatidyl Choline Liposome Coated with Pullulan bearing both a Cholesterol Moiety and a IgMs fragment," Biochem. Biophys. Acta 898: 323-330 (1987).

Large unilamellar vesicles can also be prepared in any conventional manner, such as by an extrusion procedure. Hope, M.J., et al. "Production of Large Unilamellar Vesicles by a Rapid Extrusion Procedure, Characterization of Size, Distributions, Trapped Volumes and Ability to Maintain a Membrane Potential", Biochem. Biophys. Acta 812: 55-65 (1985). Alternatively, small unilamellar vesicles can be prepared using conventional techniques such as sonication. A preferred technique for the preparation of small unilamellar vesicles is described below.

Generally, liposomes can be prepared from a variety of lipid material, including for example, phosphatidyl choline, ovolecithin, cholesterol, and steryiamino, among others. Liposomes prepared with lipid materials such as phosphatidyl choline are neutral in charge. In a preferred embodiment, however, the liposomes are prepared from lipids that will form negatively charged liposomes. Lipid materials such as, but not limited to, phosphatidyl serine, dicetyl phosphate and dimyristoyl phosphatidic acid, are examples of lipid materials that form the preferred negatively-charged liposomes. Although any charged lipid material is suitable, in a particularly preferred embodiment, phosphatidyl choline (PC) and phosphatidyl serine (PS) are combined to form negatively-charged liposomes. PC and PS may be combined in any ratio. In a particularly preferred embodiment, PC and PS are combined at a molar ratio of 1:1.

Alternatively, lipid materials such as stearylamine may be used to form positively charged liposomes.

The lipid components can be mixed with chloroform, and dried to a thin film under a stream of nitrogen. The dried lipidic material is then rehydrated. The lipidic material of the present invention is preferably rehydrated by the addition of phosphate buffered saline (pH 7.2, without $Ca2+$ or $Mg2+$) for 30 minutes at room temperature. The liposomes of the invention are formed by physically shaking the solution of rehydrated lipidic material to form small unilamellar vesicles. Although mechanical or ultrasonic shaking can be used, in a preferred embodiment, the small unilamellar vesicle type liposomes of the present invention are formed by sonication in a both sonicator for one hour and/or until no further optical clearance of the solution can be observed.

In one aspect of the present invention, the outer surface of liposome is associated with a bioactive agent such as TGF-β or M-CSF. As described above, this association refers to any kind of relationship between the parts of the association. The outer surface of the liposome may be associated by proximity to TGF-β or M-CSF so that no actual contact between the parts exists. Alternatively, the outer surface of the liposomes may be associated with TGF-β or M-CSF by superficial contact so that the outer surface of the liposome is in contact with TGF-β or M-CSF. In another embodiment, the outer surface of liposomes may

be very closely associated with TGF-β or M-CSF such that TGF-β or M-CSF is embedded, partially or fully, in a liposome. It is to be understood that, at any degree of association between TGF-β or M-CSF and the outer surface of the liposome, the association is substantially without entrapping the TGF-β or M-CSF entirely within the aqueous layer of the liposome.

It is also to be understood that the association between TGF-β or M-CSF and liposome is accomplished through means other than covalent binding. Noncovalent binding is advantageous in that, first, the maximum biological activity of the bioactive agent is required and, second, the association is achieved by simple means. In addition, noncovalent binding is advantageous in that the associated peptide can more readily dissociate from the liposome.

The association of liposomes and peptides is advantageous for several reasons. Liposomes exhibit prolonged circulation so that liposome-associated agents will circulate in the bloodstream longer than most free drugs. In fact, the time constants reported for clearance from the system range from minutes to more than a day. Longer times are reported for small vesicles made with distearoylphosphatidylcholine and sphingomyelin. J.N. Weinstein, "Liposomes in the Diagnosis and Treatment of Cancer," in Liposomes From Biophysics to Therapeutics, ed. Marc J. Ostro (1987).

In addition, association of the bioactive agents with liposomes may increase the bioavailability of the agent. The solid phase vesicles of the present invention tend to absorb to cell surfaces more than fluid vesicles, thereby enhancing presentation of the agent to the target cell.

A preferred embodiment of the present invention makes use of the ability of negatively-charged liposomes to be taken up by membrane receptors on a cell surface. Thus, these liposomes can be effective for delivery into non-phagocytic cells as well as phagocytic cells.

Another advantage of the present invention is that association of bioactive agents with liposomes may protect the bioactive agent from host enzymes and other physiological clearing mechanisms. Such protection will enhance the bioavailability of the agent.

In the present invention, the aggregates formed of both TGF-β with liposome and M-CSF with liposome exhibited biological activity. As used herein, biological activity refers to the ability of the bioactive agent to inhibit, promote or otherwise affect cellular mechanisms. As discussed above, in certain circumstances, TGF-β exhibits proliferative and antiproliferative effects, as well as effects that are unrelated to proliferation. Although the biological activity of TGF-β can be tested by other assays, the ability of TGF-β to inhibit cell growth is preferred as an indicator of biological activity. Specifically, the biological activity of TGF-β can be determined using the mink lung epithelial cell assay described in Cone et al., supra. This assay is based on the observation that the monolayer growth of the mink lung epithelial cells is inhibited in the presence of TGF-β. Details are provided in Example 3 below.

Similarly, as discussed above, the biologic activity of M-CSF refers to the ability of M-CSF to inhibit, promote or otherwise affect cellular mechanisms. Although the activity of the M-CSF of the invention may be determined in several ways, the ability of M-CSF to increase the proliferation of cells is preferred. Specifically, the biological activity of M-CSF can be determined using C3H/HeJ murine bone marrow cells in the assay as described in Strassmann, G., et al., "Regulation of Colony-Stimulating Factor 1-dependent Macrophage Precursor Proliferation by Type β Transforming Growth Factor", Journal of Immunology 140 (8):2645-2651 (1988).

The term pharmaceutically effective amount, as used herein, shall mean that amount of a bioactive agent that is effective to inhibit, promote, or otherwise affect cellular mechanisms. The term thus includes effective amounts to treat conditions such as, but not limited to, rheumatoid arthritis, systemic lupus erythematosis, autoimmune diabetes, autoimmune thyroiditis, multiple sclerosis, and other diseases. Bioactive agents may also be used to stimulate effector functions of mature monocytes such as antifungal activity and lymphokine-induced tumoricidal activity. In addition, the bioactive agents may be used in the treatment of diseases that require elevation in blood monocytes, such as leukopania. Other conditions for which bioactive agents can be used include the facilitation of allograft transplantation or the inhibition of tumor cells.

Persons of ordinary skill in the art would be able to determine the dosage of the biologically active agents of the instant invention using techniques that are known in the art. Those techniques are set out, for example, on pages 19-28 of Goodman and Gilman, The Pharmacological Basis of Therapeutics, pp. 5th Ed. (1975). Dosages can be ascertained through the use of the established assays and conventional dose-response studies. Further refinements of the calculations necessary to determine the appropriate dosage for treatment are routinely made by those of ordinary skill in the art and are within the array of tasks routinely performed by them without undue experimentation.

Appropriate pharmaceutically acceptable carriers, diluents and adjuvants can be used together with the compounds described herein to prepare the desired compositions for use in the treatment of patients. The

pharmaceutical compositions of this invention will contain the biologically active compound together with a solid or liquid pharmaceutically acceptable nontoxic carrier. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium carbonate, magnesium stearate, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol propylene glycol, water, ethanol and the like. These compositions can take the form of solutions, suspensions, tablets, pills, capsules, powders, sustained-release formulations and the like. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain an effective therapeutic amount of the active compound together with a suitable amount of carrier so as to provide the form for proper administration to the patient. While intravenous injection is a very effective form of administration, other modes can be employed, such as by injection, or by oral or parenteral administration.

The compounds of the present invention can be used in the treatment of patients. Patients shall be used in its broadest sense to mean mammals, including humans, as well as laboratory animals, for example, dogs, cats, guinea pigs, mice, and rats.

The invention will be further clarified by the following examples, which are intended to be purely exemplary of the invention.

EXAMPLE 1

PREPARATION OF LIPOSOMES, TGF-β AND TGF-β LIPOSOME AGGREGATES

A. Liposome Preparation

Small unilamellar vesicle type liposomes were prepared using Egg L-Alpha-Lecithin (Phosphatidylcholine; PC) and brain Phosphatidyl Serine (PS), purchased from Avanti Polar Lipids, Inc. (Pelham, Alabama), and radio labelled phosphatidylcholine, L-Alpha-[Dipalmitoryl-1^{14}C] - Phosphatidyl Choline (107m Ci/m Mol), purchased from New England Nuclear. Neutral liposomes were prepared using PC alone. Negatively charged liposomes were prepared by mixing PC and PS at 1:1 molar ratio.

Both PC and PC/PS lipid components were mixed with chloroform and dried to a thin film under a stream of nitrogen. Next, the two mixtures were subjected to a vacuum for one hour to remove residual traces of organic solvent. Both mixtures were then hydrated by the addition of phosphate buffered saline (PBS) (without Ca^{+2} and Mg^{+2}) at a pH of 7.2, vortex shaken for five minutes and allowed to swell for thirty minutes at room temperature. Thereafter, both mixtures were sonicated in a bath sonicator supplied by Laboratory Supplies, Hicksville, New York, for one hour or until no further optical clearance of the solution could be observed. The liposome preparations were then sterilized by filtration through a 0.2 micron polycarbonate filter. In some experiments, a trace amount of radio-labelled PC, dipalmitoyl ^{14}C phosphatidyl choline, from Dupont, was included to form radiolabelled liposomes of both types.

B. The Preparation of Transforming Growth Factor

TGF-β was purified from outdated human platelets according to a modification of the method described in Cone, et al. "An Improved Method of Purification of Transforming Growth Factor, Type Beta from Platelets," Analytical Biochemistry 168: 71-74 (1988). Following the last step of purification recited in the above reference, TGF-β in acetonitrile was aliquoted into twice siliconized glass tubes and kept frozen until use.

C. Association of TGF-β to Liposome

The twice-silliconized glass tube containing TGF-β were lyophilized for fifteen minutes to reduce acetonitrile levels. Both types of liposome preparations, neutral and negatively-charged, were added for a total volume of 0.2 ml at various lipid to protein ratios. In some experiments, a trace amount of radioactive TGF-β, ^{125}I-TGF-β, obtained from Biomedical Technologies, Inc. (Stoughton, MA) was included. Both mixtures were then incubated for one hour at 4° C with gentle agitation.

EXAMPLE 2

CHARACTERISTICS OF TGF-β-LIPOSOME AGGREGATES

A. Selection of Lipid:Protein Ratio

To determine what ratio of lipid to protein ratio yielded the maximum association of TGF-β to liposome, lipid to protein ratios of 18:1, 55:1, 167:1 and 500:1 on a w/w basis were used. As set forth in Fig. 1, the percent ^{125}I-TGF-β present in the aggregates increased from 60% at a ratio of 18:1 up to approximately 71.5% at the ratio of 167:1. At lipid to protein ratios of higher than 167:1, no significant increase in the association of TGF-β to liposome could be observed. Accordingly, the ratio 500:1 was selected for use in associating liposomes with TGF-β.

B. Efficiency and Conditions of Association

To determine the efficiency and the conditions of binding between TGF-β and the liposomes, preparations with lipid:protein ratios of 500:1 were centrifuged in a Beckman ultra centrifuge using a SW-60 rotor, at 38,000 RPM for sixteen hours in discontinuous sucrose gradients.

Radioactivity was measured in a gamma counter for the presence of ^{125}I. The activity of TGF-β was determined by an inhibition assay using mink lung epithelial cells (CCL64) as indicators as described in Cone et al. supra.

As set forth in Table 1, migration patterns of ^{125}I-TGF-β indicated association of TGF-β with PC/PS liposome. The migration pattern of ^{125}I-TGF-β in preparations of neutral PC liposomes was similar to that exhibited by ^{125}I-TGF-β in preparations where no lipids were present (category designated "NONE"), indicating that, rather than associating and migrating with neutral PC liposomes, TGF-β migrated throughout the gradient as an unassociated protein. In contrast, when negatively-charged PC/PS liposome preparations were employed, TGF-β exhibited a tight migration pattern and migrated into the 5%, 10%, and 20% levels. The presence of biologically active TGF-β at each of those levels was determined by mink lung epithelial cell (CCL-64) assay to be the greater than 94.5 ng. In contrast, no activity level greater than 21 ng was found in any band in the gradients using TGF-β and PC liposomes. These results suggest that the presence of ^{125}I-TGF-β corresponded to increased TGF-β activity in the PC/PS preparation.

In addition to exhibiting a tight migration pattern in a discontinuous sucrose gradient, TGF-β associated with PC/PS liposomes exhibited very little non-specific binding to the ultracentrifuge tube compared to TGF-β with PC liposomes or TGF-β alone. Specifically, as set forth in the lower part of Table 1, only 8% of ^{125}I-TGF-β in presence of PC liposomes or TGF-β alone. Thus, the adherance of the TGF-β molecules appeared to be greatly diminished when associated with PC/PS liposomes.

TABLE I

DISTRIBUTION OF TGF-BETA ASSOCIATED WITH LIPOSOMES IN DISCONTINUOUS SUCROSE GRADIENT					
LIPID COMPOSITION		PC/PS		PC	NONE
% SUCROSE	%^{125}I-TGF-β	ACTIVITY (ng)	%^{125}I-TGF-β	ACTIVITY (ng)	%^{125}I-TGF-β
0	4.3	2.27	4.5	6.8	7.8
5	9.2	94.5	8.8	9.45	18.0
10	26.5	94.5	10.1	20.5	15.2
20	41.5	94.5	12.6	21	12.2
40 a	4.4	21.0	8.8	21	3.6
40 b	4.4	15.0	8.8	19.95	3.6
IN ULTRA CENTRIFUGE-TUBE	8.0	N.D.	41	N.D.	40

* LIPID TO PROTEIN RATIO 500 : 1. 38000 RPM, 16 HOURS in SW-60 ROTOR

C. Distribution of TGF-β Liposomes on Sucrose Gradients

In a second set of experiments, a continuous sucrose gradient was used according to the method of Baxter-Baggard, "A Simple Method for the Large-Scale Preparation of Sucrose Gradients," FEBS Letters, 20(1): 117-119 (January 1972). The gradient was obtained by subjecting 20% sucrose in PBS to three rounds of freezing and thawing. Approximately 300 ul of TGF-β liposome were loaded onto four ml gradients which were then centrifuged at 100,000 times g at 4° centigrade for eighteen hours in the ultra centrifuge.

At the end of the centrifugation, 0.2 ml fractions were collected from the top the gradient. Radioactivity was measured by liquid scintillation counter for ^{14}C and in a gamma counter for ^{125}I. The amount of biologically active TGF-β in each fraction was determined by inhibition assay using mink lung epithelial cells as indicators and known amounts of TGF-β as a standard, according to the method described in Cone, et al., supra.

Figures 2 and 3 demonstrate the association of TGF-β to liposome preparations made up of PC/PS lipids and lack of association of TGF-β to liposome preparations made up of only PC liposomes.

In both figures, three measurements were made for each fraction:

^{14}C-PC - indicating presence of PC component of liposomes;

^{125}I-TGF-β - indicating presence of TGF-β; and

TGF-β activity - confirming presence of TGF-β and indicating biological activity of TGF-β.

In Figure 2, where only PC is employed as liposome constituent, presence and activity of TGF-β were independent of presence of liposomes. Throughout all ten fractions, presence of TGF-β ranged from about 10 to 14 cpm x 10^3 and activity of TGF ranged from 0 to 75 ng/fraction. In contrast, in Figure 3, where negatively-charged PC/PS liposomes were employed, both presence of TGF-β and activity of TGF-β were highly correlated to presence of PC/PS liposomes. Specifically, fraction 4, which contained the highest level of negatively-charged liposomes, also contained highest level of TGF-β, 25 x 10^3 CPM, and exhibited greatest TGF-β activity, approximately 925 ng. Accordingly, it is demonstrated that TGF-β associates with negatively charged PC/PS liposomes and not with neutral PC liposomes.

EXAMPLE 3

DETERMINATION OF BIOLOGIC ACTIVITY IN VITRO

Because TGF-β is known to suppress the stimulation of mink lung epithelial cells, designated MV-1-LU cells (American Type Culture Collection CCL-64 cells (hereinafter CCL-64 cells)) that action was employed to determine biologic activity in vitro. Specifically, the ability of TGF-β and TGF-β associated liposomes were analyzed for the ability to inhibit proliferation of these cells. The extent of inhibition was determined by tritiated thymidine incorporation.

Mink lung epithelial cells (American Type Tissue Collection CCL64) maintained in Dulbecco's modified Eagles' medium (GIBCO), ("DMEM") plus 10% fetal calf serum (HyClone) are known to be released by trypsin. 0.1 ml of cells containing 2 x 10^4 ml were seeded into 96-well tissue culture plates. Aliquots of TGF-β standard or samples to be assayed were then added to the plates. After a 24 hour incubation at 37°C ub a 5% CO_2 atmosphere, the wells were pulsed with 25 μl of 40 μCi/ml tritiated thymidine (New England Nuclear, 6.7 Ci/mM). Approximately 16 hours later, the wells were washed three times with DMEM and incubated for 15-20 minutes with trypsin to release the cells. The cells were then collected on glass paper by a semi-automated cell harvester (Skatron). Using the same bioassay, a standard curve with known amounts of TGF-β was constructed. The amount of active TGF-β in a given sample was determined by comparison of the inhibition obtained to the standard curve.

As set forth in Table II, the percentage inhibition was measured for three treatments: PC/PS liposomes alone, for TGF-β associated with PC/PS liposomes, and for TGF-β alone. As set forth in the Constant Presence portion of Table II when the three solutions were allquoted directly into the tissue culture plates and kept at a constant presence, both TGF-β and TGF-β associated with liposomes exhibited high percentages of inhibition, 82% and 90%, respectively, in contrast to 0% inhibition by liposomes alone. Thus, the biological activity of TGF-β associated with liposomes corresponds to the biological activity of TGF-β alone.

To test the effect of association of TGF-β with liposome on absorption by cells, the cells were then subjected to sequential transfer. In this study, identical cultures of cells exposed to the three treatments were sequentially transferred at thirty minute intervals into fresh tissue culture plates.

The cells that were exposed to liposome alone exhibited no inhibition after each transfer. The pattern of inhibition varied, however, for cells that were exposed to TGF-β with and without liposomes. For cells exposed to TGF-β alone, the cells of the first transfer exhibited essentially the same inhibition as that demonstrated with constant presence. That inhibition decreased to a significant degree, however, with the second transfer. Specifically, cells exposed to only TGF-β exhibited a percentage inhibition of 83% in the first transfer but only 35% in the second transfer. In the third, fourth, fifth, and end transfer, the cells exhibited no inhibition. Accordingly, it appeared that the TGF-β molecules were absorbed by the cells in the first and second transfer so that the cells of the later transfers did not contain enough TGF-β to show any anti-proliferative effects.

In contrast, the cells that were exposed to TGF-β associated with liposomes exhibited another pattern of inhibition. Although the cells of the first transfer exhibited an inhibition of 54%, this may be attributed to leakage of TGF-β from the cells. The significance lies in the inhibition exhibited by the cells of the end transfer. That percentage of inhibition, 81%, at the end of transfer is similar to the inhibition seen with cells exposed to a constant presence of TGF-β. Accordingly, it appeared that the liposomes inhibited adherance of TGF-β to the cells such that TGF-β was sequentially transferred and able to exhibit its antiproliferative effect in the end transfer.

TABLE II

TGF-β LIPOSOMES DELIVER A NON-ABSORBABLE NEGATIVE SIGNAL FOR SERUM STIMULATED CCL-64			
PERCENTAGE OF INHIBITION			
TREATMENT	LIPOSOMES	TGF-β LIPOSOMES (1.25ng/ml)	TGF-β (1.25ng/ml)
CONSTANT TRANSFER	0	90	82
FIRST TRANSFER (30 MIN)	0	54	83
SECOND TRANSFER (30 MIN)	0	0	35
THIRD TRANSFER (30 MIN)	0	0	0
FOURTH TRANSFER (30 MIN)	0	0	0
FIFTH TRANSFER (30 MIN)	0	0	0
END TRANSFER	0	81	0
CONTROL = 57081 CPM			

EXAMPLE 4

THE ABILITY OF TGF-β-LIPOSOME AGGREGATES TO SUPPRESS THE IMMUNE REACTION AGAINST C. PARVUM IN VIVO.

Because the spleen functions to filter blood, bacterial infection often leads to nonspecific acute splenitis which results in an enlargement of the spleen and an increase in the number of lymphocytes within the spleen. The ability of TGF-β to inhibit proliferation of spleen cells otherwise stimulated by bacterial infection was employed as an in vivo assay for the activity of TGF-β associated with liposomes.

In this assay, the strain of mice known as C57BL/6 were inoculated intraperitonially with 0.2 ml of PBS containing either 0.7 or 1 mg heat-killed Corynobacterium parvum (obtained from Welcome Research Laboratories, Research Triangle Park, North Carolina). Twenty-four hours later, the mice received an intravenous injection of 0.2 ml of PBS containing (a) 1 µg of TGF-β, (b) 0.5 mg PC/PS liposome, or (c) 1 µg of TGF-β associated with 0.5 mg PC/PS liposomes (1:500 ratio). These solutions were not subjected to sucrose gradients. The treatment was repeated three times at daily intervals.

Six to eight days after immunization with bacteria, the mice were sacrificed. The spleens were weighed and single cell suspensions were analyzed to determine the extent of the immune response against the bacteria. The results are presented in Table III.

Based on the anti-proliferative properties of TGF-β, it was expected that spleens of mice that received 1 µg TGF-β associated with 0.5 mg PC/PS liposomes would not exhibit either increased size or cell number. In contrast, it was expected that spleens of mice that received either only 1 µg TGF-β or 0.5 mg PC/PS liposomes would exhibit the enlarged spleen cell and increased spleen cell number of an advanced immune response.

In Experiment I, mice that had been treated with TGF-β associated with liposomes yielded a mean spleen weight of 109 ± 12 milligrams and 232 x 10^6 cells per spleen. These data corresponded to an inhibition of the immune response of 82%. In contrast, mice that had received only TGF-β, without liposomes, exhibited spleen weights of 165 ± 30 milligrams and 308 x 10^6 cells per spleen for an inhibition of 44%. Mice treated with only liposomes exhibited spleen weights of 224 ± 10 milligrams and 440 x 10^6 cells per spleen, which indicated percent inhibition of only 10%.

In Experiment II, mice which had received TGF-β associated with liposomes yielded spleen weights of 139 ± 18, and 227 x 10^6 cells per spleen, which indicated an inhibition of 92%. This figure is nearly three times that of TGF-β injected mice where spleen weights of 172 ± 57 milligrams and cell numbers of 342 x

10^6 cells per spleen were obtained for an inhibition rate of 38%.

TABLE III

EFFECT OF TGF-BETA ASSOCIATED WITH LIPOSOMES ON ACUTE SPLENITIS IN C. PARVUM CHALLENGED C57BL/6 MICE

INJECTION OF C. PARVUM	TREATMENT	SPLEEN WEIGHT (MG)*	# CELLS PER SPLEEN (x 10^6)	% INHIBITION
		EXPERIMENT I		
-	-	72 ± 3	179	-
+	PBS	239 ± 103	466	-
+	LIPOSOMES	224 ± 10	440	10 %
+	TGF-BETA	165 ± 30	308	44 %
+	TGF-BETA & LIPOSOMES	109 ± 18	227	92 %
		EXPERIMENT II		
-	-	106 ± 33	211	-
+	LIPOSOMES	210 ± 30	417	-
+	TGF-BETA	172 ± 57	342	36 %
+	TGF-BETA & LIPOSOMES	139 ± 18	227	92 %

* AVERAGE OF 3 MICE ± S.D.

EXAMPLE 5

DELAYED TYPE HYPERSENSITIVITY (DTH) TO LISTERIA MONOCYTOGENESIS

As described in Kaufmann and Hahn, "Biological Functions of T-Cell Lines with Specificity for the Intracellular Bacterium Listeria monocytogenes In-Vitro and In-Vivo," J. Exp. Med. 155: 1754-1765 (June 1982), experimental infection of mice with Listeria monocytogenesis has been widely used to study cellular immune response to bacteria. In a double blind study, the strain of mice known as C57B1/6 were injected with live bacteria at a concentration of 5 x 10^4. Five or six days later, the spleens of those mice were removed and used to prepare a single cell suspension. After removal of adherent cells, the nonadherent splenocytes at a concentration of 25 x 10^6 were injected intravenously into syngeneic recipient mice, i.e., mice that were genetically identical to the initial injected mice with respect to immune reactions. Sixteen hours later, these later injected mice were divided into three or four groups. One group received 0.2 ml PBS containing 1 µg TGF-β; another received PBS with 0.5 mg PC/PS liposome, the third group received 1 µg TGF-β associated with 0.5 mg PC/PS liposomes (1:500 ratio), and the fourth, where present, received 0.2 ml PBS alone.

One hour later, the right foot pad of each mouse was injected with 50 ul of PBS containing 5 x 10^7 heat-killed Listeria, and, as a control, the left foot was injected with 50 µl PBS. Foot pad swelling was measured 24 hours later with a micrometer. A 0.01 mm difference in thickness between the Listeria-injected and PBS injected foot pad was taken as one DTH unit.

The difference in foot pad swelling, as indicated by DTH units, corresponds to the effect of TGF-β on the immune response. Specifically, the antiproliferative effect of TGF-β manifests as decreased foot pad swelling and fewer DTH units. As set forth in Figures 4 and 5, the presence of liposomes associated TGF-β caused a significant decrease in foot swelling. Specifically, compared to liposomes alone (LIP), TGF-β along (TGF-β), or a control (CONT), TGF-β associated with liposomes (LT) exhibited significantly fewer DTH units. In both Figs. 4 and 5, the decrease in DTH units for TGF-β liposomes (LT), represents a 50% reduction in

the Immune response with a statistical difference of ($P < 0.01$). Accordingly, TGF-β associated with liposomes exhibited in vivo reduction in the immune response.

EXAMPLE 6

PREPARATION OF LIPOSOMES, M-CSF, AND M-CSF LIPOSOME AGGREGATES

A. Positively-Charged Liposome Preparation

Small unllamellar vesicle type liposomes were prepared using 10 mg of Egg L-Alpha-Lecithin (Phosphatidylcholine; PC) purchased from Avanti Polar Lipids, Inc. (Pelham, Alabama), 0.5 µCi radio labelled phosphatidylcholine, L-Alpha-[Dipalmitoryl-114C] -Phosphatidyl Choline (107m Ci/m Mol), purchased from New England Nuclear, and 2.5 mg of stearylamine purchased from Sigma.

Neutral liposomes (for controls) were prepared as above except without stearylamine.

The lipid components were mixed with chloroform in a glass tube and dried to a thin film under a stream of nitrogen. Next, the two mixtures were lyophilized for 30 minutes to remove residual traces of organic solvent. Both mixtures were then hydrated by the addition of phosphate buffered saline (PBS) (without Ca+2 and Mg+2) to a concentration of 5 mg/ml liquid at a pH of 7.2, vortex shaken for five minutes and allowed to swell for thirty minutes at room temperature. Thereafter, both mixtures were sonicated in a bath sonicator supplied by Laboratory Supplies, Hicksville, New York, three times, at approximately 20 minutes each time, at room temperature. The liposome preparations were then sterilized by filtration through a 0.2 micron polycarbonate filter.

B. The Preparation of Macrophage Colony-Stimulating Factor

The M-CSF was obtained by the renaturation and purification of human truncated M-CSF expressed in E. coli.

1. Construction and Expression

The expression of a truncated form of human M-CSF was performed using two cistronic expression systems. The COS expression plasmid designated pcDhMCSF11-185, which encodes N-terminal 185 amino acid residues of the 554 amino acid M-CSF precursor was digested with ScaI and BamHi restriction enzymes. The resultant fragment (about 450 bp) was ligated with a synthetic linker which possessed an internal SD sequence, a termination codon for the first cistron and an initiation codon for the second cistron. The ligated fragment was inserted between the XbaI and BamHi sites of prepIL-2D8 . The resultant plasmid, ptrpII-2X M-CSF101, coding for a 151 amino acid sequence of M-CSF was then transformed into E. coli by the CaCl2 method. Such recombinant techniques are provided in the copending U.S. Application Serial No. 07/304,692, filed February 1, 1989, entitled "Human Colony-Stimulating Factors," to Takahashi, M. et. al.

2. Renaturation

The transformed E. coli cells were shaken in supplemented M9 medium which also contained ampicillin. The cells were harvested and pelleted and washed with Triton X-100. The final pellet contained an inclusion body containing the truncated M-CSF. The pellet was solubilized in 7.0 M guanidin hydrochrolide and 25mM 2-mercaptoethanol with stirring for 4 hours at room temperature. The solubilized pellet was slowly dropped into 2,000 ml of glutathione solution (0.5 mM reduced, 0.1 mM oxidized glutathione and 2.0 M urea in 50 mM Tris/HCL, ph 8,5) with intense stirring. The solution was kept at 4°C for 48 hours.

After 10 ml of the renatured solution was concentrated to 500ul in an Amicon membrane, the solution was applied at a rate of 0.7 ml/min to a gel filtration HPLC on Shodex WS-803 column previously

equilibrated with 40 mM sodium phosphate containing 0.3 M NaCl, ph 6.8. The fractions were collected and assayed for M-CSF activity.

3. Purification

The renatured solution was centrifuged and the supernatant applied to QAE-ZeTA Prep Cartridge 100 from Pharmacia-LKB which was previously equilibrated with 50 mM Tris/HCl at ph 8.5 and eluted with 0.5 M NaCl in 50 mM Tris/HCl, pH 8.5.

After ammonium sulfate precipitation, the supernatant was applied to a TSK-gel Phenyl-5PW HPLC column (Toso, 21.5 x 1500 mm), preequilibrated with 40 mM sodium phosphate, pH 7.4 and containing a saturated ammonium sulfate solution. The active fragments were obtained at 6-3% ammonium sulfate and concentrated and exchanged against 40 mM sodium phosphate, pH 7.4.

The concentrated sample was applied to a TSK-gel DEAE 5PW column (Toso, 21.5 x 150 mm) preequilabrated with 40 mM sodium phosphate, pH 7.4, and eluted at a flow rate of 3 ml/mln by a NaCl gradient.

C. Preparation of Liposome M-CSF Aggregates

The combinations of liposome and M-CSF set forth in Table VI below were mixed together, vortexed, incubated at room temperature for 5 minutes.

TABLE VI

Addition Tube #	C^{14}-PC/S Liposomes	C^{14}-PC Liposomes	M-CSF	^{125}I-M-CSF
1	500 ^mgm (100 ^ mli)	--	5ugm (5 ^mli)	0.1 ^mgm (6X10)^{5}cpm In
2	500 ^mgm (100 ^ mli)	--	5ugm (5 ^mli)	--
3	500 ^mgm (100 ^ mli)	--	--	--
4	--	500 ^mgm	5 ^mgm	0.1 ^mgm
5	--	500 ^mgm	5 ^mgm	--
6	--	500 ^mgm	--	--
7	--	--	5 ^mgm	0.1 ^mgm

EXAMPLE 7

CHARACTERISTICS OF M-CSF-LIPOSOME AGGREGATES

A. Preparation of Sucrose Gradients

To form sucrose gradients, 1 ml of a 20% sucrose solution was added to the bottom of polyallomer tubes 1-7. On the top of each layer, 1 ml aliquots of 10% and 5% and 2.5% sucrose were carefully layered to form final volumes of 4 ml In each of the tubes.

The liposome-M-CSF mixtures prepared in Example 6 (c) above were loaded on the top of the appropriately numbered sucrose gradient. The tubes were centrifuged in a Beckman ultracentrifuge for 16 hrs at 40000 rpm in SW-60 rotor. At the end of centrifugation, 0.2 ml fractions were collected from the top of the gradient and counted for radioactivity of 14C and 125I. The levels of 14C indicated the relative concentration of liposome in the fractions. Similarly, the level of 125I indicated the relative concentration of M-CSF in the fractions. After the fractions were filter sterilized, the activity of M-CSF in each fraction was

determined against a M-CSF standard curve in the assay set forth below.

B. Assay for M-CSF Activity

The C3H/HeJ murine bone marrow cells which proliferate in response to M-CSF were used to assay the activity of M-CSF. The cells were seeded in flat-bottomed microtiter plates (Costar, Cambridge, Ma) at a density of 1 x 105. The mixtures of liposome and M-CSF were serially diluted and added to the plates for a final volume of 0.2 ml.

The cells were cultured for three days. Eight hours before the end of the culture period, cells were pulsed with 1µCi of [methyl-3H] TdR (6.7 Ci/mmol; New England Nuclear Boston, Ma). The incorporation of [methyl-3H] TdR into the cells was determined by standard liquid scintillation counting procedures after harvest of cells using a semiautomatic cell harvester (Skatron, Sterling, Va).

The extent of proliferation in a given fraction was extrapolated from M-CSF dose curves prepared under the same conditions. Strassman, G., et al., "Regulation of Colony-Stimulating Factor 1-dependent Macrophage Precursor Proliferation by Type β Transforming Growth Factor", Journal of Immunology 140 (8):2645-2651 (1988); Moore, R.N., et al., "Endogenous Regulation of Macrophage Proliferative Expansion by Colony-Stimulating factor-induced Interferon," Science 223: 178 (1984); and Pullen, S.K. et al., "Bone Marrow-derived Macrophage Expression of Endogenous and Transfected Class II MHC Genes during Differentiation In Vitro," Journal of Immunology 137: 1359 (1986).

C. Efficiency and Conditions of Association

As set forth in Table VII below, the migration patterns of 125I indicate association of M-CSF with positively charged liposomes. The migration pattern of M-CSF in preparations of neutral PC liposomes (PC - M-CSF In Tubes 4 and 5) was similar to that exhibited by M-CSF in preparations without liposomes (M-CSF only in Tube 7), indicating that, rather than associating and migrating with neutral liposomes, M-CSF migrated throughout the gradient as an unassociated protein. In contrast, when positively-charged PC/S liposome preparations were employed (PC/S - M-CSF in Tubes 1 and 2), M-CSF exhibited a tight migration pattern and predominated in fractions 2-6, the same fractions into which PC/S liposomes predominated, both in the presence of M-CSF (Tubes 1 and 2) and in the absence of M-CSF (Tube 3, PC/S only).

This study also showed that biologically active M-CSF associated with positively-charged liposomes. The presence of biologically active M-CSF in each fraction was determined with C3H/HeJ murine bone marrow cells as described above. In preparations of M-CSF alone (Tube 7) and M-CSF with neutral PC (Tubes 4 and 5), fractions 6-9 contained biologically active M-CSF. The presence of neutral liposome seemed to have no impact on the presence of biologically active M-CSF. In contrast, in preparations of M-CSF with positively-charged liposomes (PC/C -M-CSF in Tubes 1 and 2), those fractions in which liposomes predominated were the same fractions in which both presence and activity of M-CSF were greatest. Thus, it appears that the presence of positively charged liposomes affected the migration of biologically active M-CSF and suggests that M-CSF exhibits preferential association with positively-charged liposomes.

Table VII

Association of M-CSF (Recombinant Human) with Preformed Liposomes with Opposite Charge

	PC/S - M-CSF			PC/S Only		PC - M-CSF			PC Only	M-CSF Only		
Tube #	1	2		3		4	5		6	7		
Fraction From Top	^{14}C	^{125}I	Activity (ug)	^{14}C	Activity (ug)	^{14}C	^{125}I	Activity (ug)	^{14}C	^{125}I	Activity (ug)	% Sucrose
1	722	34851		689		34	12090		58	9926		
2	4322*	216658*	0.4	5546*	N.D.	29	14160		29	9863		
3	2401*	112926*		2374*		49	21190		110	9698		2.5%
4	2322*	116803*	1.6	2285*	N.D.	63	26933		153	10914		
5	1275*	112835*		999		98	54933		226	28858		
6	1225*	133836*		362		182	103423*		304	89008*		
7	603	130666*		266		281	160693*		666	118213*	0.4	
8	429	120998*		261		2180*	140677*	1.5	2711*	123342*		5.0%
9	322	83688		203		3106*	110186*		3417*	73695*	0.8	
10	182	50430		142		3833*	49588		5272*	57940		
11	113	33304		130		3821*	23318		2912*	16416		
12	130	21857		75		2005	10289		1544	6905		
13	85	13073		66		581	5531		1187	4703		10%
14	84	7333		95		896	4319		832	2402		
15	29	5149		29		502	2125		391	1201		
16	36	3750		98		362	566		255	714		
17	55	2372		34		220	388		104	441		
18	107	2012		66		92	306		63	307		20%
19	62	1050		66		50	204		50	106		
20	60	561		60		60	200		49	107		

Lipid Protein Ration 100:1 (500 ug lipid; 5 ug M-CSF Ec-80-I) 40000 rpm, 16 hrs, SW-60 rotor 19° C
N.D. Not Detected

*represents peak

Other embodiments of the invention will be apparent to those skilled in the art from a consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

Claims

1. A method of treating a patient with bioactive agents, comprising:

(a) providing aggregates of bioactive agents in association with liposomes, wherein the aggregates comprise

i. liposomes having outer surfaces; and

ii. bioactive agents;

wherein the bioactive agent is associated with the outer surfaces of the liposome such that the biological activity of the bioactive agent is not substantially diminished when the aggregates are administered in a pharmaceutically effective amount to a patient, and

(b) administering the aggregates to the patients.

2. The method of claim 1, wherein the bioactive agents are selected from the group consisting of TGF-β and M-CSF.

3. The method of claim 1, wherein the liposomes are selected from the group consisting of small

unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles.

4. The method of claim 3, wherein the liposomes are small unilamellar vesicles.

5. The method of claim 4, wherein the small unilamellar vesicles are comprised of phosphatidyl choline and phosphatidyl serine.

6. The method of claim 5, wherein the phosphatidyl choline and phosphatidyl serine are in a molar ratio of 1:1.

7. The method of claim 4 wherein the small unilamellar vesicles are comprised on phosphatidyl choline and stearylamine.

8. A composition of matter, comprising:

aggregates of a bioactive agent in association with liposomes, wherein the aggregates comprise:

(a) a multiplicity of liposomes having outer surfaces; and

(b) a multiplicity of bioactive agents;

wherein the bioactive agent is associated with the outer surfaces of the liposome such that biological activity of the bioactive agent is not substantially diminished when the aggregates are administered in a pharmaceutically effective amount to a patient.

9. The composition of claim 8, wherein the bioactive agent is selected from a group consising of TGF-β and M-CSF.

10. The composition of claim 8, wherein the liposomes are selected from a group comprising small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles.

11. The composition of claim 10, wherein the liposomes are small unilamellar vesicles.

12. The composition of claim 11, wherein the small unilamellar vesicles are comprised of phosphatidyl choline and phosphatidyl serine.

13. The composition of claim 12, wherein the phosphatidyl choline and phosphatidyl serine are in a molar ratio of 1:1.

14. The composition of claim 11, wherein the small unilamellar vesicles are comprised of phosphatidyl choline and stearylamine.

15. A pharmaceutical composition comprising:
(a) a pharmaceutically effective amount of the composition of claim 8, and
(b) a pharmaceutically acceptable carrier.

16. A pharmaceutical composition comprising:
(a) a pharmaceutically effective amount of the composition of claim 9, and
(b) a pharmaceutically acceptable carrier.

17. A pharmaceutical composition comprising:
(a) a pharmaceutically effective amount of the composition of claim 10, and
(b) a pharmaceutically acceptable carrier.

18. A pharmaceutical composition comprising:
(a) a pharmaceutically effective amount of the composition of claim 11, and
(b) a pharmaceutically acceptable carrier.

19. A pharmaceutical composition comprising:
(a) a pharmaceutically effective amount of the composition of claim 12, and
(b) a pharmaceutically acceptable carrier.

20. A pharmaceutical composition comprising:
(a) a pharmaceutically effective amount of the composition of claim 13, and
(b) a pharmaceutically acceptable carrier.

21. A pharmaceutical composition comprising:
(a) a pharmaceutically effective amount of the composition of claim 14, and
(b) a pharmaceutically acceptable carrier.

22. A method of treating a patient with a bioactive agent, comprising:

(a) providing aggregates of bioactive agents in association with liposomes, wherein the aggregates comprise:

i. charged liposomes having outer surfaces; and

ii. a bioactive agent, having a net charge that is the complement of the charge on the liposomes;

wherein the bioactive agent is associated with the outer surfaces of the liposome such that the biological activity of the bioactive agent is not substantially diminished when the aggregates are administered in a pharmaceutically effective amount to a patient, and

(b) administering the aggregates to the patients.

23. The method of claim 22, wherein the liposomes bear a net negative charge and the bioactive compound bears a net positive charge.

24. The method of claim 23, wherein the bioactive compound is TGF-β.

25. The method of claim 22, wherein the liposomes bear a net positive charge and the bioactive agent bears a net negative charge.

26. The method of claim 25, wherein the bioactive agent is M-CSF.

27. The method of claim 22, wherein the charged liposomes are selected from the group consisting of small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles.

28. The method of claim 27, wherein the liposomes are small unilamellar vesicles.

29. The method of claim 28, wherein the small unilamellar vesicles are comprised of phosphatidyl choline and phosphatidyl serine.

30. The method of claim 29, wherein the phosphatidyl choline and phosphatidyl serine are in a molar ratio of 1:1.

31. The method of claim 28, wherein the small unilamellar vesicles are comprised of phosphatidyl choline and stearylamine.

32. A composition of matter, comprising:

aggregates of bioactive agents in association with liposomes, wherein the aggregates comprise

(a) a multiplicity of charged liposomes having outer surfaces; and

(b) a multiplicity of bioactive agents, having a charge that is the complement to the charge on the liposomes;

wherein the bioactive agent is associated with the outer surfaces of the liposome such that biological activity of the bioactive agent is not substantially diminished when the aggregates are administered in a pharmaceutically effective amount to a patient.

33. The composition of matter of claim 32, wherein the liposome is negatively charged and the bioactive agent is positively charged.

34. The composition of matter of claim 33, wherein the bioactive agent is TGF-β.

35. The composition of matter of claim 32, wherein the liposomes are positively charged and the bioactive agent is negatively charged.

36. The composition of matter of claim 35, wherein the bioactive agent is M-CSF.

37. The composition of matter of claim 32, wherein the charged liposomes are selected from the group consisting of small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles.

38. The composition of matter of claim 37, wherein the liposomes are small unilamellar vesicles.

39. The composition of matter of claim 38, wherein the small unilamellar vesicles are comprised of phosphatidyl choline and phosphatidyl serine.

40. The composition of matter of claim 39, wherein the phosphatidyl choline and phosphatidyl serine are in a molar ratio of 1:1.

41. The composition of matter of claim 38, wherein the small unilamellar vesicles are comprised of phosphatidyl choline and stearylamine.

42. A pharmaceutical composition comprising:
(a) a pharmaceutically effective amount of the composition of claim 32, and
(b) a pharmaceutically acceptable carrier.

43. A pharmaceutical composition comprising:
(a) a pharmaceutically effective amount of the composition of claim 33, and
(b) a pharmaceutically acceptable carrier.

44. A pharmaceutical composition comprising:
(a) a pharmaceutically effective amount of the composition of claim 34, and
(b) a pharmaceutically acceptable carrier.

45. A pharmaceutical composition comprising:
(a) a pharmaceutically effective amount of the composition of claim 35, and
(b) a pharmaceutically acceptable carrier.

46. A pharmaceutical composition comprising:
(a) a pharmaceutically effective amount of the composition of claim 36, and
(b) a pharmaceutically acceptable carrier.

47. A pharmaceutical composition comprising:
(a) a pharmaceutically effective amount of the composition of claim 37, and
(b) a pharmaceutically acceptable carrier.

48. A pharmaceutical composition comprising:
(a) a pharmaceutically effective amount of the composition of claim 38, and
(b) a pharmaceutically acceptable carrier.

49. A pharmaceutical composition comprising:

(a) a pharmaceutically effective amount of the composition of claim 39, and
(b) a pharmaceutically acceptable carrier.

50. A pharmaceutical composition comprising:
(a) a pharmaceutically effective amount of the composition of claim 40, and
(b) a pharmaceutically acceptable carrier.

51. A pharmaceutical composition comprising:
(a) a pharmaceutically effective amount of the composition of claim 41, and
(b) a pharmaceutically acceptable carrier.

52. The use of an aggregate comprising a bioactive agent in association with the outer surface of a liposome for the manufacture of a pharmaceutical.

Figure 1

The Effect of Varying Lipid/Protein Ratios on the Association of TGF-β With Negatively-Charged Liposomes

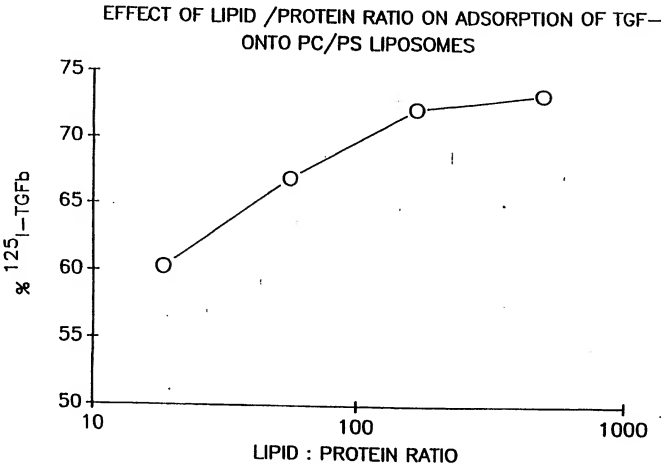

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Figure 2

The Extent of TGF-β Association with Neutral Liposomes

PC LIPOSOMES

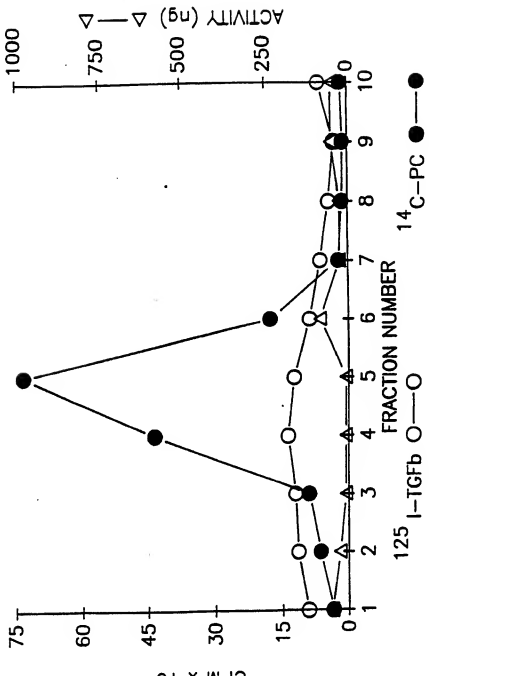


Figure 3

The Extent of TGF-β Association With Negatively-Charged Liposomes

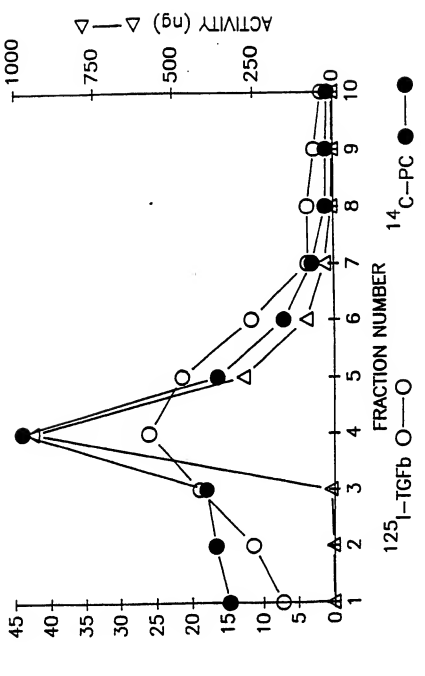


Figure 4

The Effects of TGF-β Associated Liposomes on Delayed Type Hypersensitivity Responses to Listeria

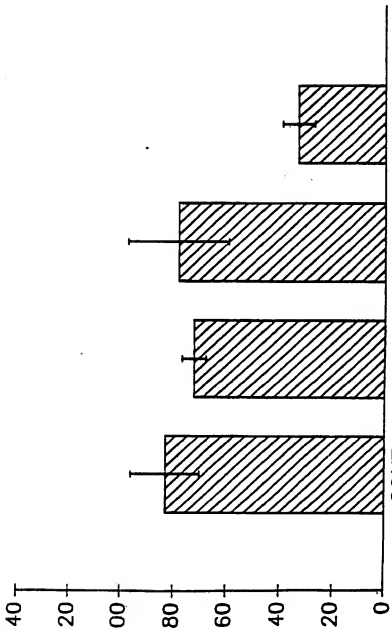
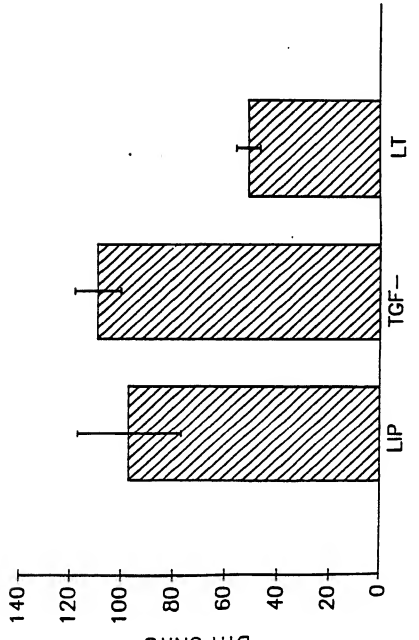


Figure 5

The Effects of TGF-β Associated Liposomes on Delayed Type Hypersensitivity Responses to Listeria



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(71) Applicant: **L'OREAL SA**
(72) Inventor: **RIBIER ALAIN**
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ARNAUD-SEBILLOTTE LAURENCE
SEGOT EVELYNE

(54) DEPIGMENTING COMPOSITION FOR SIMULTANEOUS TREATMENT OF SURFACE LAYER AND DEEP LAYER OF SKIN, ITS USE AND SKIN DEPIGMENTATION

(57) Abstract:

PURPOSE: To obtain a depigmenting composition intended to make two kinds of active agents act simultaneously on both the surface and deep layers of the skin and markedly raise the efficacy of the composition and the complementary or synergistic effect of these active agents.

CONSTITUTION: This depigmenting composition comprises a 1st dispersion of lipid vesicles A capable of penetrating into the deep layers of the skin and containing an active agent selected from the group consisting of anti-pigmenting agents, depigmenting agents and tyrosinase inhibitors, for treating these deep layers, and a 2nd dispersion of lipid vesicles B capable of penetrating into the surface layers of the skin and containing an active agent selected from the group consisting of keratolytic agents, moisturizing agents and protective agents, for treating these surface layers. The vesicles A enable the ASL in the stratum corneum to be diffused at a rate of $>1\times10^{-7}$ cm^2s^{-1}, being provided with glucose encapsulating ability of $\geq$24 h, while the vesicles B enable the AS in the stratum corneum to be diffused at a rate of $<1\times10^{-7}$ cm^2s^{-1} being provided with glucose encapsulating ability of 24 h. At room temperature, the vesicles A are in a fluid state, while the vesicles B gelled state.

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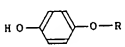
(72) Inventor: **KANEKI HIROYUKI**
YAMAGUCHI MICHIHIRO
MACHIDA YASUHIKO
AKIYASU AKIRA

(54) LIPOSOME PHARMACEUTICAL

(57) Abstract:

PURPOSE: A liposome pharmaceutical, obtained by embedding a hydroquinone glycoside on a lamella phase of a complex lipid. and incorporating sterol as a stabilizer therein and having improved stability and selective migration to the affected part of the hydroquinone glycoside and further sustained release properties.

CONSTITUTION: A liposome pharmaceutical obtained by embedding a hydroquinone glycoside expressed by the formula (R is residue, e.g. L-arabinose) in a lamella phase of a complex lipid, e.g. natural or synthetic phospholipid, at 1:0.2W0.7 weight ratio of the former to the latter, and incorporating sterol, e.g. cholesterol or β-sitosterol, as a stabilizer therein. A charge is desirably imparted to the lamella phase of the complex lipid for enhancing the dispersion stability of the liposome. When a negative charge is imparted, a lipid, e.g. phosphatidyl serine or dicetyl phosphate, having the negative charge is incorporated. When a positive charge is imparted, a lipid, e.g. stearylamine, having the positive charge is incorporated.



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Preparation for the transport of an active substance across barriers

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(54) Title: PREPARATION FOR THE TRANSPORT OF AN ACTIVE SUBSTANCE ACROSS BARRIERS

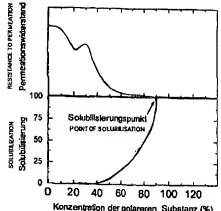
(54) Bezeichnung: PRÄPARAT ZUM WIRKSTOFFTRANSPORT DURCH BARRIEREN

(57) Abstract

Preparation for the application of an active substance in the form of minute droplets, especially liquid droplets with a membrane-type sheath of at least one or more layers of amphiphilic molecules or an amphiphilic carrier substance, especially for the transport of an active substance into and through natural barriers and constrictions such as skin and the like. The preparation has no point of solubilization or the preparation composition is at maximum permeation capacity far from solubilization point. The preparation contains at least two components whose solubility in suspending agents of the preparations, generally water, differs by at least a factor of 10.

(57) Zusammenfassung

Präparat zur Wirkstoffapplikation in Form kleinster Tröpfchen, insbesondere mit einer membranartigen Hülle aus einer oder wenigen Lagen amphiphiler Moleküle bzw. mit einer amphiphilen Trägersubstanz versehenen Flüssigkeitströpfchen, insbesondere zum Transport des Wirkstoffes in und durch natürliche Barrieren und Konstriktionen wie Haute und dergleichen. Das Präparat weist keinen Solubilisierungspunkt auf oder die Präparatzusammensetzung ist bei maximaler Permeationsfähigkeit weit vom Solubilisierungspunkt entfernt. Das Präparat weist einen Gehalt von mindestens zwei Komponenten auf, die sich in ihrer Löslichkeit im Suspensionsmedium der Präparate, üblicherweise Wasser, um mindestens de Faktor 10 unterscheiden.



Preparation For Transporting Active Ingredients Through Barriers

The invention relates to new preparations for the administration of active ingredients in the form of very small liquid droplets, which can be suspended in a liquid medium, have a membrane-like sheath of one or a few layers of molecules, comprise an active ingredient and, in particular, are suitable for transporting the active ingredient through barriers, such as natural permeability barriers and constrictions in skin, mucous membranes, organs and the like.

Moreover, the invention relates to a method for the production of such preparations, especially for the non-invasive administration of active ingredients.

The administration of active ingredients frequently is limited by natural barriers, such as the skin, which prevent adequate introduction of active ingredients, since they are not sufficiently permeable to the active ingredients. For example, because of the permeability barrier of the skin, most current therapeutic agents must be administered perorally or parenterally (i.v., i.m., i.p.). Intrapulmonal and intranasal applications of aerosols, the use of rectal suppositories, the application of gels to mucous membranes, ocular preparations, etc. can be realised only at certain places and not with all active ingredients. The introduction of active ingredients into vegetable tissue is subject to even greater limitations because of the cuticular wax layers.

Non-invasive administrations of active ingredient preparations, which are suitable for penetrating such permeability barriers, would be advantageous in many cases. In man and animal, for example, a percutaneous administration of such preparations would protect the active ingredients administered against decomposition in the gastrointestinal tract and possibly result in a modified distribution of the agent in the body; it can affect the pharmacokinetics of the drug and permit a frequent as well as a simple non-invasive treatment (Karzel, K., Liedtke, R.K. (1989) Arzneim. Forsch./Drug Res. 39, 1487 - 1491). In the case of plants, improved penetration through or into the cuticle could lower the concentration of active ingredient required for the desired effect and, in addition, could significantly decrease contamination of the environment (Price, C.E. (1981) in: The Plant Cuticle (D.F. Cutler, K.L. Alvin, C.E. Price, Publisher), Academic, New York, pp. 237 - 252).

Efforts to influence skin permeability by suitable measures have been discussed frequently (see, for example, Karzel and Liedtke, op. cit.). Especially worth mentioning are, for example, Jet injection (Siddiqui & Chien (1987) Crit. Rev. Ther. Drug. Carrier, Syst. 3, 195 - 208), the use of electrical fields (Bumette & Ongpipattanakul (1987) J. Pharm. Sci. 76, 765 - 773) or the use of chemical additives, such as solvents or surfactants. A long list of inactive ingredients, which were tested for the purpose of increasing the penetration of a water-soluble active ingredient (Nolaxon) into the skin, is contained, for example, in the work of Aungst et. al. (1986, Int. J. Pharm. 33, 225 -234).

The best-known method for increasing penetration of active ingredient through the skin or mucous membrane is based on the use of penetration enhancers. Such penetration enhancers comprise nonionic materials (long-chain alcohols, surfactants, zwitterionic phospholipids), anionic materials (particularly fatty acids), cationic long-chain amines, sulfoxides, as well as various amino derivatives, and amphoteric glycinates and betaines. Nevertheless, the problem of the penetration of active ingredient into the skin has not yet been solved or not yet been solved satisfactorily.

An overview of the measures, which have been used for the purpose of increasing active ingredient penetration through plant cuticles, is summarised in the work of Prince (1981, op. cit.).

The penetration enhancers, which have previously exclusively been used exclusively, increase the ability to penetrate the permeability barrier of the skin or mucous membrane surface, in that they increase the fluidity of a portion of the lipids in this barrier. When chemical penetration enhancers were used, it has previously been customary to add these simply to a mixture containing the active ingredient; only in the case of human skin were additives sometimes also applied especially in the form of an organic solution. This form of administration was associated with the previously investigated and discussed principles of action of additives. In general, it was assumed that the increased penetration of the agent, on the one hand, is based on the softening (fluidisation) of the skin (Golden et. al. (1987) I. Pharm. Sci. 76, 25 - 28). As a rule, the softening of the skin is associated with a destruction of the skin surface and its protecting barrier properties and consequently is undesirable. On the other hand, it was shown that some active ingredients permeate through the skin in the form of low molecular weight complexes with the additive molecules (Green et. al. (1988) Int. J. Pharm. 48, 103 - 111).

Proposals, deviating from these concepts, such as the epidermal use of lipid suspension, have brought about little improvement until now. Such suspensions typically contain vesicles or O/W or W/O emulsifiers.

The percutaneous use of carriers on a lipid basis, the liposomes (Patel, Bloch. Soc. Trans., 609th Meeting, 13, 513 - 517, 1985, Mezei, M. Top. Pharm. Sci. (Proc. 45th Int. Congr. Pharm. Sci. F.I.P.) 345 - 58 Elsevier, Amsterdam, 1985), which was discussed theoretically by several authors, was directed mainly at influencing the kinetics of the active ingredient. There was discussion of the use of conventional lipid vesicles, which pass through the skin extremely incompletely, if at all, as shown in this patent application. The use of liposomes, niosomes or other conventional lipid vesicles is therefore limited to external layers of the skin.

In a similar sense, the Japanese patent application JP 61/271204 A2 (86/27 1204) took up the use of liposomes by using hydroquinone glucosidal as a material, which increases the stability of the active ingredient.

The use of lipid vesicles carrying the active ingredient, together with a gel-forming agent, in the form of "transdermal patches" was proposed as an improvement in the WO 87/1938 Al. In this way, it was possible to prolong the period of action; however, the ability of the active ingredient to permeate was hardly increased. By the massive use of penetration-promoting polyethylene glycol and fatty acids together with lipid vesicles, Gesztes and Mezei (1988, Anesth. Analg. 67, 1079 - 1081) succeeded in attaining local analgesia with lidocaine-containing carriers, however, only after several hours of occlusive application and on a small scale.

Furthermore, carrier formulations were found, which are suitable for penetrating into and through permeability barriers. For example, it was possible for the first time to surpass the results of Gesztes and Mezei dramatically by a special formulation, which contained filtered, detergent-containing lipid vesicles (liposomes) with a declared optimum lipid/surfactant content of 1 - 40/1 and, in practice, generally of 4/1.

Furthermore, it was recognised that all such carriers, which are sufficiently elastic in order to be able to penetrate through the constriction of the barrier, such as of the skin, are suitable for penetrating into and through permeability barriers. This is so particularly if the carriers, after the application itself, build up a gradient at the permeability barrier, since in this case they tend to penetrate the permeability barrier spontaneously. In the DE 41 07 152 and DE 41 07 153 patent applications, carriers, which are referred to in the following as transfersomes, are described for the first time; they are useful for transporting active ingredients through almost any permeation barrier.

Transfersomes differ from the liposomes, previously described for topical use, and from other carriers used with respect to several basic properties. As a rule, transfersomes are much larger than conventional micelle-like carrier formulations and are therefore subject to different diffusion laws. For example, the permeability is not a linear function of the driving pressure, as it is in the case of liposomes, that is, in the case of transfersomes, the permeability, in contrast to liposomes or other known similar carrier systems, increases disproportionately or nonlinearly as the pressure increases. Furthermore, substances introduced through constrictions by means of transfersomes, can develop in man almost 100% of the maximum obtainable biological or therapeutic potential. For example, more than 50% and frequently more than 90% of the active ingredients, which have been applied percutaneously and packaged in transfersomes, regularly reach their site of destination in the body. These transfersomes, described in the EP 91114 163 and PCT/EP 91/01596, contain a boundary-active substance, which corresponds up to 99 mole percent of the content and at least 0.1 mole percent of this substance, by means of which the solubilising point of the droplets is attained.

The content of boundary-active substance, which brings about an optimised approximation of the solubilisation limit of the transfersomes (that is, a content of boundary-active substance, which destabilises the transfersomes completely), so that they are sufficiently elastic in order to be able to penetrate through constrictions in the barrier, such as those in the skin, was stated to be the decisive condition for the ability of the transfersomes to penetrate, which is greater than that of the liposomes or of similar known carriers.

For the formulation of such high-grade preparations capable of permeating, it would now be highly desirable not to be bound by the content ranges named.

It is therefore an object of the invention to indicate transfersomes for the administration of active ingredients, which transfersomes either do not have a solubilisation point or are far removed from the solubilisation point and permit the rapid and effective transport of active ingredients through barriers and constrictions.

It is furthermore an object of the invention to make available transfersomes for the transport of active ingredients through human, animal and vegetation barriers, which transfersomes make possible the improved availability of the active ingredient at the site of action.

It is furthermore an object of the invention to indicate a method for the preparation of such transfersomes for transporting active ingredients.

The distinguishing features of the independent claims serve to accomplish this objective.

Advantageous developments are given in the dependent claims.

Surprisingly, it was found that it is also possible to form transfersome preparations, which are suitable for the administration or transport of at least one active ingredient, especially for medical and biological purposes, into and through natural barriers and constructions, such as skin and the like, and have the form of liquid droplets, which can be suspended in a liquid medium and are provided with a membrane-like sheath of one or a few layers of amphiphilic carrier substance, the carrier substance comprising at least two amphiphilic components, which are physically and/or chemically different and differ in their solubility in the suspension medium of the transfersomes (usually water), by a factor of at least 10, if their content of solubilising components amounts to less than 0.1 mole percent based on the content of these substances, for which the solubilising point of the enveloped droplets is reached or the amphiphilic components are selected so that, independently of the concentration, there is no solubilisation at all of the enveloped droplets.

The inventive preparations, referred to in the following once again as transfersomes, can be prepared from any amphiphilic components, which have sufficiently different solubilities. This condition is fulfilled, if the solubilities of the individual carrier components of the transfersome in the suspension medium differ at least by a factor of 10 (and of up to 10^7). Fulfilling this condition ensures that the membrane-like sheath of the resulting transfersomes, under the influence of a gradient such as an intact natural barrier like the skin, has an increased deformability. This property enables the inventive transfersomes to penetrate through the constrictions in any permeability barriers.

The ability of the inventive preparations to permeate through constrictions is at least 0.001 percent and, preferably, more than 0.1 percent of the permeability of small, essentially unimpeded, permeated molecules.

According to present knowledge (but without having to be bound to a theoretical, scientific definition), the concept of solubility, as used here, refers to so-called true solutions. In any case, when a limiting concentration is reached, a solubility limit is observed, which is defined by the formation of a precipitate, the formation of crystals, the formation of suspensions or by the formation of molecular aggregates, such as micelles. For self-aggregating molecules, the solubility limit typically corresponds to the critical self-aggregation concentration (CAC). For molecules forming micelles, the solubility limit typically corresponds to the critical micelle concentration (CMC).

The inventive transfersomes differ appreciably from the previously described transfersomes. In particular, the transfersomes of the present application differ from known transfersomes owing to the fact that the transfersomes can be formed from combinations of any components, irrespective of their solubilising capability.

Moreover, the stability of the inventive transfersomes is even better than that of the known transfersomes (see patent applications WO 92703122 and EP 475 160), since the transfersomes composition is not close to the solubilisation point.

Figure 1 shows the decrease in the permeation resistance at a barrier as a function of the concentration of boundary-active substance with respect to the approach to the solubilisation point for transfersomes described in the state of the art (this solubilisation point, however, not being reached).

Figure 2 shows, for inventive transfersomes, the decrease in the permeation resistance at a barrier as a function of the component concentration with respect to the approach to a theoretical solubilisation point, which cannot be reached in practice.

Figure 2 clearly shows that, for the component system of the inventive transfersomes, there is no solubilisation point or the solubilisation point is still far away when the maximum permeation capability is reached.

The inventive transfersomes accordingly open up an elegant, uniform and generally useful path for the transport of various active ingredients into or through permeability barriers. This newly discovered carrier for active ingredients is suitable for use in human and veterinary medicine, dermatology, cosmetics, biology, biotechnology, egricultural technology and in other areas.

A transfersome furthermore is distinguished by its ability to penetrate or diffuse under the action of a gradient through and/or into permeability barriers and, in so doing, transports materials, particularly active ingredients. This ability can easily be recognised and quantified owing to the fact that the curve, for which the permeation capability is plotted as a function of gradient, is not linear.

Pursuant to the invention, such a transfersome is composed of several to many molecules, which form a unit physicochemically, physically, thermodynamically and frequently functionally. The optimum transfersome size is a function of the barrier characteristics. It depends on the polarity (hydrophilicity), mobility (dynamics) and charge, as well as on the elasticity of the transfersome (surface). The size of a transfersome advantageously is between 10 and 10 000nm.

Pursuant to the invention, transfersomes, preferably having a size of 50 to 10 000nm, frequently of 75 to 400nm and particularly of 100 to 200nm, are used for dermatological applications.

For applications to plants, mostly relatively small transfersomes, predominantly with a diameter smaller than 500nm, advisably are used.

The vesicle radius of the preparation droplets (transfersomes) is approximately 25 to 500, preferably 50 to 200 and particularly 80 to 180nm.

For inventive transfersomes of any amphiphilic materials, preferably one or more components with a water solubility between 10^{-10} M and 10^{-6} M and one or more components with a water solubility between 10^{-6} M and 10^{-3} M are combined. Alternatively, the amphiphilic components, which can be combined, can be assigned to one another also on the basis of their HLB values, the difference between the HLB values of the two components preferably amounting up to 10 and frequently being between 2 and 7 and, particularly, between 3 and 5.

The penetration capability of the inventive transfersomes can be determined by measurements, in which they are compared with reference particles or molecules. The reference particles used are clearly smaller than the constrictions in the barriers and accordingly have maximum permeation capability. Preferably, the permeation rate of transfersomes through a test barrier ($P_{transfer}$), when the barrier itself is the site of the determination, should not differ by more than a factor of 10^{-1} to 10^{-3} from the permeation rate of the comparison materials P_{refer} (such as water). If a relatively uniform and slow transport of material through the barrier is desired, the given ratio should lie between 10^{-4} and 1. The permeation capability is at a maximum, when the ratio of $P_{transfer}/P_{refer}$ is greater than 10^{-2}. This data refers to transfersomes, which are larger than the constrictions by more than a factor of 2 and less

than a factor of 4. With increasing size difference between carrier and constrictions, that is, when the factor is greater than 4, the $P_{transfer}/P_{refer}$ values can be correspondingly smaller.

Transfersomes of this application may consist of one or several components. Most frequently, a mixture of basic substances is used. Suitable basic substances comprise lipids and other amphiphilic substances, as well as hydrophilic liquids; these can be mixed with the active ingredient molecules in particular ratios, which depend on the choice of substances as well as on their absolute concentrations.

In general, the preparations contain at least two amphiphilic components of different solubility for forming a membrane-like sheath around an amount of droplet of a hydrophilic liquid, the active ingredient being contained in the membrane-like sheath, for example, a double membrane and/or in the hydrophilic liquid. The association between active ingredient and carrier may also take place at least partially only after the formation of transfersome-like droplets.

If the transfersomes inherently are not adequately deformable and their permeation capability is to be attained by the addition of boundary-active materials, the concentration of these materials corresponds to less than 0.1 mole percent of the amount, which would be required for solubilising the transfersomes, or this solubilisation is not attainable at all in the practically relevant concentration range.

The inventive transfersomes are useful for transporting active ingredients through almost any permeation obstacle, for example, for a percutaneous administration of a drug. They can transport water-soluble, amphiphilic or fat-soluble agents and, depending on their composition, on the amount applied and on their form, attain different depths of penetration. The special properties, which make a carrier out of a transfersome, can be attained by phospholipid-containing vesicles as well as by other amphiphilic aggregates. For example, a large proportion of active ingredient molecules can be carried not only into the barrier, for example, into the skin, but also through the barrier by means of such transfersomes and consequently become systemically active. For example, transfersomes carry polypeptide molecules through the skin 1000 times more efficiently than was previously possible with the help of permeation-promoting structureless materials.

Definitions:

Lipids:

In the sense of this invention, a lipid is any substance, which has properties like or similar to those of a fat. As a rule, it has an extended apolar group (the chain, X) and generally also a water-soluble, polar hydrophilic part, the head group (Y) and has the basic formula 1.

$$X - Y_n \quad (1)$$

wherein n is equal to or larger than zero. Lipids with n = 0 are referred to as apolar lipids and lipids with n > 1 are referred to as polar lipids. In this sense, all amphiphilic substances, such as glycerides, glycerophospholipids, glycerophosphinolipids, glycerophosphonolipids, sulfolipids, sphingolipids, isoprenoid lipids, steroids or sterols and carbohydrate-containing lipids can generally be referred to as lipids.

A phospholipid is, for example, a compound of formula 2:

$$R\text{-}CH_2\text{-}CHR_2\text{-}CR_3H\text{-}POO_R\text{-}O\text{-}R_4 \times G^+ \quad (2)$$

wherein n and R_4 have the meanings given under formula 2, R_1, R_2 cannot be hydrogen, OH or a short-chain alkyl group and R_3 generally is hydrogen or OH. Furthermore, R_4 is a short-chain alkyl group, substituted by a tri-short-chain alkylammonium group, such as a trimethylammonium group, or an amino-substituted short-chain alkyl group, such as 2-trimethylammonium ethyl group (cholinyl).

A lipid preferably is a substance of formula 2, wherein n = 1, R_1 and R_2 are hydroxyacyl, R_3 is hydrogen and R_4 is 2-trimethylammonium ethyl (the latter corresponds to the phosphatidyl choline head group), 2-dimethylammonium ethyl, 2-methylammonium ethyl or 2-aminoethyl (corresponding to the phosphatidyl ethanolamine head group).

Such a lipid is, for example, a natural phosphatidyl choline, which used to be called lecithin. It can be obtained from egg (rich in arachidonic acid), soybean (rich in C_{18} chains), coconut (rich in saturated chains), olives (rich in monounsaturated chains), saffron (safflower) and sunflowers (rich in n-6 linoleic acid), linseed (rich in n-3 linolenic acid), from whale fat (rich in monounsaturated n-3 chains), from primrose or primula (rich in n-3 chains). Preferred, natural phosphatidyl ethanolamines (used to be called cephalins) frequently originate from egg or soybeans.

Furthermore, synthetic phosphatidyl cholines (R_4 in formula 2 corresponds to 2-trimethylammoniumethyl), synthetic phosphatidyl ethanolamines (R_4 is 2-aminoethyl), synthetic phosphatid acids (R_4 is a proton) or its ester (R_4 corresponds, for example, to a short-chain alkyl, such as methyl or ethyl), synthetic phosphatidyl serines (R_4 is L- or D-serine), or synthetic phosphatidyl (poly)alcohols, such as phosphatidyl inositol, phosphatidyl glycerol (R_4 is L- or D-glycerol) are preferred as lipids, wherein R_1 and R_2 are identical acyloxy groups, such as lauroyl, oleoyl, linoyl, linoleoyl or arachinoyl, such as dilauroyl, dimyristoyl, dipalmitoyl, distearoyl, diarachinoyl, dioleoyl, dilinoyl, dilinolenyl, diliniolyl, dilinolinoyl, dilininolenoyl or diarachinoyl phosphatidyl choline or ethanolamine, or various acyl groups, such as R_1 = palmitoyl and R_4 = oleoyl, such as l-palmitoyl-2-oleoyl-3-glycerophosphocholine, or various hydroxyacyl groups, such as R_1 = hydroxypalmitoyl and R_4 = oleoyl etc. Moreover, R_1 can represent alkenyl and R_2 identical hydroxyalkyl groups, such as tetradecylhydroxy or hexadecylhydroxy, for example, in ditetradecyl or dihexadecylphosphatidyl choline or ethanolamine, R_1 can represent alkenyl and R_2 hydroxyacyl, such as a plasmalogen (R_4 trimethylammonium ethyl), or R_1 can be acyl, such as lauryl, myristoyl or palmitoyl and R_2 can represent hydroxy as, for example, in natural or synthetic lysophosphatidyl cholines or lysophosphatidyl glycerols or lysophosphatidyl ethanolamines, such as 1-myristoyl or 1-palmitoyllysophosphatidyl choline or -phosphatidyl ethanolamine; frequently, R_3 represents hydrogen.

A lipid of formula 2 is also a suitable lipid within the sense of this invention. In formula 2, n = 1, R_1 is an alkenyl group, R_2 is an acylamido group, R_3 is hydrogen and R_4 represents 2-trimethylammonium ethyl (choline group). Such a lipid is known under the name of sphingomyelin.

Suitable lipids furthermore are a lysophosphatidyl choline analog, such as 1-lauroyl-1,3-dihydroxypropane-3-phosphoryl choline, a monoglyceride, such as monoolein or monomyristin, a cerebroside, ceramide polyhexoside, sulfatide, sphingoplasmalogen, a ganglioside or a glyceride, which does not contain a free or esterified phosphoryl or phosphono or phosphino group in the 3 position. An example of such a glyceride is diacylglyceride or 1-alkenyl-1-hydroxy-2-acyl glyceride

with any acyl or alkenyl groups, wherein the 3-hydroxy group is etherified by one of the carbohydrate groups named, for example, by a galactosyl group such as a monogalactosyl glycerin.

Lipids with desirable head or chain group properties can also be formed by biochemical means, for example, by means of phospholipases (such as phospholipase A1, A2, B, C and, in particular, D), desaturases, elongases, acyl transferases, etc., from natural or synthetic precursors.

Furthermore, a suitable lipid is any lipid, which is contained in biological membranes and can be extracted with the help of apolar organic solvents, such as chloroform. Aside from the lipids already mentioned, such lipids also include, for example, steroids, such as estradiol, or sterols, such as cholesterol, β-sitosterol, desmosterol, 7-keto-cholesterol or β-cholestanol, fat-soluble vitamins, such as retinoids, vitamins, such as vitamin A1 or A2, vitamin E, vitamin K, such as vitamin K1 or K2 or vitamin D1 or D3, etc.

The less soluble amphiphilic components comprise or preferably comprise a synthetic lipid, such as myristoleoyl, palmitoleoyl, petroselinyl, petroselaidyl, oleoyl, elaidyl, cis- or trans-vaccenoyl, linolyl, linolenyl, linolaidyl, octadecatetraenoyl, gondoyl, eicosaenoyl, eicosadienoyl, eicosatrienoyl, arachidoyl, cis- or trans-docosaenoyl, docosadienoyl, docosatrienoyl, docosatetraenoyl, lauroyl, tridecanoyl, myristoyl, pentadecanoyl, palmitoyl, heptadecanoyl, stearoyl or nonadecanoyl, glycerophospholipid or corresponding derivatives with branched chains or a corresponding dialkyl or sphingosin derivative, glycolipid or other diacyl or dialkyl lipid.

The more soluble amphiphiilc components(s) is/are frequently derived from the less soluble components listed above and, to increase the solubility, substituted and/or complexed and/or associated with a butanoyl, pentanoyl, hexanoyl, heptanoyl, octanoyl, nonanoyl, decanoyl or undecanoyl substituent or several, mutually independent, selected substituents or with a different material for improving the solubility.

A further suitable lipid is a diacyl- or dialkyl-glycerophosphoethanolamine azo polyethoxylene derivative, a didecanoylphosphatidyl choline or a diacylphosphooligomaltobionamide.

Within the sense of this invention, any other substance (such as a poly- or oligoamino acid), which has a slight or at least regionally a slight solubility in polar materials, is regarded as a lipid.

All surfactants and asymmetric, and therefore amphiphilic molecules or polymers, such as some oligocarbohydrates and polycarbohydrates, oligopeptides and polypeptides, oligonucleotides and polynucleotides, many alcohols or derivatives of such molecules belong to this category.

The polarity of the "solvents", surfactants, lipids or active ingredients depends on the effective, relative hydrophilicity/hydrophobicity of the respective molecule. However, it also depends on the choice of other system components and boundary conditions in the system (temperature, salt content, pH, etc.). Functional groups, such as double bonds in the hydrophobic group, which weaken the hydrophobic character of this group, increase the polarity; extensions of or bulky substituents in the hydrophobic group, such as in the aromatic group, lower the polarity of a substance. Charged or highly polar groups in the head group, while the hydrophobic chain remains the same, normally contribute to a higher polarity and solubility of the molecules. Direct bonds between the lipophilic and/or amphiphilic system components have the opposite action.

In particular, all compounds named in the EP patent application 475 160 as being boundary active, are suitable as highly polar substances. The disclosure of this patent application is herewith explicitly referred to.

Active ingredients:

The inventive transfersomes are suitable for the administration of the most different active ingredients, particularly, for example, for therapeutic purposes. For example, inventive preparations may contain, in particular, all the active ingredients named in the EP patent application 475 160.

Furthermore, inventive preparations may contain, as active ingredient, an adrenocorticostatic agent, a β-adrenolytic agent, an androgen or antiandrogen, an anti-parasitic, anabolic, anaesthetic or analgesic, analeptic, anti-allergic, anti-arrhythmic, anti-arteriosclerosis, anti-asthmatic and/or bronchospasmolytic agent, an antibiotic, an anti-depresstve and/or anti-psychotic agent, an anti-diabetic agent, an antidote, an anti-emetic, anti-epileptic, anti-fibrinolytic, anti-convulsive or anti-cholinergic agent, an enzyme, coenzyme or a corresponding inhibitor, an antihistamine, an antihypertensive drug, a biological activity inhibitor, an anti-hypotensive drug, an anticoagulant, an anti-mycotic or antimyasthenic agent, an active ingredient against Parkinson's or Alzheimer's disease, an anti-phlogistic, anti-pyretic or anti-rheumatic agent, an antiseptic, a respiratory analeptic or stimulating agent, a broncholytic, cardiotonic or chemotherapeutic agent, a coronary dilator, a cytostatic agent, a diuretic, a ganglion blocker, a glucocorticoid, a therapeutic agent for influenza, a haemostatic or hypnotic agent, immunoglobulin or fragment or a different immunological or receptor substance, a bioactive carbohydrate (derivative), a contraceptive, a migraine agent, a mineral corticoid, a morphine antagonist, a muscle relaxant, a narcotic, a neural or CNS therapeutic agent, a nucleotide or polynucleotide, a neuroleptic agent, a neuron transmitter or a corresponding antagonist, a peptide (derivative), an ophthalmic agent, a (para)-sympathicomimetic or (para)-sympathicolytic agent, a protein (derivative), a psoriasis/neurodermatitis agent, a mydriatic agent, a mood elevator, a rhinological agent, a sleeping draft or its antagonist, a sedative, a spasmolytic, tuberculostatic or urological agent, a vasoconstrictor or dilator, a virostatic agent or a wound-healing agent or several such agents.

Preferably, the active ingredient is a non-steroidal anti-inflammatory drug, such as diclofenac, ibuprofen or a lithium, sodium, potassium, caesium, rubidium, ammonium, monomethyl, dimethyl, trimethytanimonium or ethylammonium salt thereof.

Moreover, the inventive preparations may contain, as active ingredient, a growth-regulating substance for living beings, a biocide, such as an insecticide, pesticide, herbicide, fungicide or an attureent, particularly a pheromone.

As less polar components, inventive preparations may contain a physiologically compatible lipid, preferably from the class of phospholipids and especially from the class of phosphatidyl cholines, the active ingredient, for example, ibuprofen, diclofenac or a salt thereof, being the more soluble component, optionally with the addition of less than 10% by weight, based on the total composition of the preparation of a further soluble component and the concentration of the more soluble component(s) typically being between 0.01% by weight and 15% by weight, preferably between 0.1% and 10% by weight and particularly between 0.5% by weight and 3% by weight, and the total lipid

concentration being between 0.005% by weight and 40% by weight and preferably between 0.5% by weight and 15% by weight and especially between 1% by weight and 10% by weight.

Inventive preparations additionally may comprise consistency modifiers, such as hydrogels, antioxidants such as probucol, tocopherol, BHT, ascorbic acid, desferroxamine and/or stabilisers such as phenol, cresol, benzyl alcohol, etc.

Unless specified otherwise, all the substances indicated, surfactants, lipids, active ingredients or additives with one or more chiral carbon atoms can be used either as racemic mixtures or as optically pure enantiomers.

Principle of Action:

In the case of permeation barriers, the transport of the active ingredients can be accomplished by those transfersomes, which satisfy the following basic criteria:

- The transfersomes shall sense or build up a gradient, which drives them into or over the barrier, for example, from the body surface into and under the skin, from the leaf surface into the interior of the leaf, from one side of the barrier to the other;
- The resistance to permeation, which the transfersomes sense in the barrier, shall be as small as possible in comparison to the driving force;
- The transfersomes shall be able to permeate into and/or through the barrier, without at the same time losing the enclosed active ingredients in an uncontrolled manner.

Furthermore, the transfersomes shall permit the distribution and effects of the active ingredient and the course of action as a function of time to be controlled. If necessary, they shall also be able to bring the material into the depth of the barrier and beyond the barrier and/or to catalyse such a transport. Last but not least, the transfersomes shall have an effect on the range and depths of action, as well as, in favourable cases, the nature of the cells, the tissue parts, the organs or the system sections, which are reached or treated.

In a first respect, the chemical gradients come into consideration for the biological applications. Particularly suitable are the physicochemical gradients, such as the (de)hydration pressure (moisture gradient) or a concentration difference between the site of application and the site of action; however, electrical or magnetic fields, as well as thermal gradients are of interest in this respect. For technical applications, the hydrostatic pressure applied or an existing pressure difference is furthermore of importance.

In order to fulfit the second condition, the transfersomes must be sufficiently "liquid" on the microscopic scale, that is, they must have a sufficiently high mechanical elasticity and deformability and a sufficiently low viscosity; only then can they pass through the constrictions within the permeability barrier.

Understandably, the resistance to permeation decreases with carrier size. However, the driving force frequently also depends on the carrier size; if the pressure is independent of size, this force typically decreases with size. For this reason, the transfer coefficient is not a simple function of size and frequently has a maximum, which depends on the choice of carrier and active ingredient.

Furthermore, the choice of carrier substance, active ingredients and additives, as well as the amount or concentration of carrier applied play a role. A low dosage generally leads to a surface

treatment. At the same time, materials of low water solubility generally remain in the apolar region of the permeability barrier (for example, in the membranes of the epidermis). Readily soluble active ingredients, which diffuse easily out of the carriers, may have a distribution different from that of the carrier. For such materials, the permeability of the transfersome membrane is also of importance. Substances, which tend to cross over from the carriers into the barrier, lead to a locally variable carrier composition, etc. These relationships should be considered and taken into consideration before any application. When searching for conditions, under which simple carrier vesicles become transfersomes, the following rule of thumb can be used:

- To begin with, two or more amphiphilic components are combined, which differ in their solubility in the intended suspension medium of the transfersomes, usually water or a different polar, generally aqueous medium by a factor of 10 to 10^7 preferably of 10^2 to 10^6 and especially of 10^3 to 10^5, the less soluble component having a solubility of 10^{-10} to 10^{-6} and the more soluble component a solubility of 10^{-6} to 10^{-3} M. The solubility of the corresponding components, if not known from general, conventional reference works, can be determined, for example, by conventional methods of determining the saturation limit.

- As a next step up, the carrier composition or concentration of the components in the system is adapted, so that the vesicles are sufficiently stable as well as adequately deformable, and therefore have appropriate permeation capability. In this application, stability is understood to be mechanical "coherence" as well as the fact that the substance content and, in particular, the active ingredient content of the carrier composition does not change or does not change significantly during the transport and particularly not during the permeation process. The position of the optimum sought depends on the components selected.

- Finally, the system parameters are optimised, taking into consideration the application methods and the objectives aimed for. For a rapid action, a high permeation capability is required; for a slow release of active ingredient, a gradual penetration of the barrier and a correspondingly adjusted membrane permeability are advantageous; for action at a depth, a high dose is advisable and for as wide a distribution as possible, a carrier concentration that is not too high.

- The content of amphiphilic components is adjusted, in particular, so that the ability of the transfersomes preparation to permeate through constrictions is at least 0.001 percent of the permeability of small molecules (for example, water). The ability of the inventive transfersomes to penetrate can be determined by means of measurements, in which the transfersomes are compared with reference particles or molecules. The reference particles used are clearly smaller than the constrictions in the barrier and thus have maximum permeation capability. Preferably, the difference between the transfersome permeation rate through a test barrier ($P_{transfer}$) and the permeation rate of the comparison materials (P_{refer}) (such as water) should not be greater than a factor of 10^{-5} to 10^{-1} when the barrier itself is the site of the determination.

In this application, relevant properties of the transfersomes as carriers for the lipid vesicles are discussed. Most of the examples refer, by way of example, to phospholipids as carrier. However, the general validity of the conclusions is not limited to this class of carriers or these molecules. The lipid vesicle examples merely illustrate the properties, which are required for penetration through the

permeability barriers, such as the skin, for example. The same properties also make possible the transport of a carrier through the animal or human epidermis, mucous membranes, plant cuticles, inorganic membranes, etc.

The probable reason for the spontaneous permeation of transfersomes through the "pores" in the layer of corneal corpuscles is the fact that one side of these pores ends in an aqueous compartment, the subcutaneous tissue; for this permeation, the transfersomes are driven by osmotic pressure. Alternatively, however, an external pressure, such as a hydrostatic or an electro-osmotic pressure can be applied additionally.

Depending on the amount of vesicles, the lipid vesicles can reach as far as the subcutaneous tissue after a percutaneous application. The active ingredients, depending on the size, composition and formulation of the carrier or agents, are released locally, accumulated proximally or passed on over lymph or blood vessels and distributed over the body.

It is sometimes appropriate to adjust the pH of the formulation immediately after the production or immediately before use. Such an adjustment is intended to prevent the destruction of the components of the system and/or of the active ingredient carriers under the initial pH conditions and to ensure the physiological compatibility of the formulation. For the neutralisation, physiologically compatible acids or bases and buffer solutions with a pH of 3 to 12, preferably of 5 to 9 and especially of 6 to 8, depending on the purpose and site of the application, are generally used. Physiologically compatible acids are, for example, dilute aqueous mineral acids, such as dilute hydrochloric acid, sulfuric acid or phosphoric acid, or organic acids such as alkane carboxylic acids like acetic acid. Physiologically compatible alkalis are, for example, dilute sodium hydroxide solution, appropriately ionised phosphoric acid, etc.

The preparation temperature is normally adapted to the substances used and, for aqueous preparations, usually is between 0°C and 95°C. Preferably, the temperature ranges from 18°C to 70°C; for lipids with fluid chains, the temperature range preferably is between 15°C and 55°C and, for lipids with ordered chains, between 45°C and 60°C. Other temperature ranges are possible for non-aqueous systems or preparations, which contain cooling or heating preservatives, or which are prepared in situ.

If required by the sensitivity of the components of the system, the formulations can be stored cool (for example, at 4°C). They can also, however, be prepared and stored under the atmosphere of an inert gas, such as nitrogen. The shelf life can be increased further by using substances without multiple bonds, as well as by drying and using dry substance, which is dissolved and worked up only on the spot. In particular, the transfersomes-like droplets can be prepared from a concentrate or a lyophilisate shortly before use.

In most cases, the carriers are applied at room temperature. Use at lower temperatures or at higher temperatures, with synthetic substances at even higher temperatures, is entirely possible.

A transfersomes suspension can be produced by means of supplying mechanical, thermal, chemical or electrical energy. For example, the preparation of a transfersome can be based on homogenising or stirring.

The formation of transfersome-like droplets can be brought about by filtration. The filter material, which can be used for this purpose, should have a pore size of 0.01 to 0.8µm, especially of 0.05 to 0.3µm and particularly of 0.08 to 0.15µm. Optionally, several filters can be arranged in series.

The preparations can be prepared in advance or at the site of use, as described, for example, in P 40 26 83 3.0-43 or by means of several examples in the handbook "Liposomes" (G. Gregoriadis, published by CRC press, Boca Raton, FL, volumes 1 to 3, 1987), in the book "Liposomes as Drug Carriers" (G. (Gregoriadis, published by John Wiley & Sons, New York, 1988), or in the laboratory handbook "Liposomes. A Practical Approach" (R. New, Oxford Press, 1989). If necessary, an active ingredient suspension can be diluted or concentrated immediately before use, for example, (by ultracentrifugation or ultrafiltration) or mixed with further additives. For this, however, the possibility, that the optimum for the carrier permeation will be shifted, must be excluded or taken into consideration.

The transfersomes of this application are suitable as carriers of lipophilic materials, such as fat-soluble biological active ingredients, therapeutic agents and poisons, etc.; their use in connection with amphiphilic, water-soluble substances is also of great practical value, particularly when their molecular weight is greater than 1000.

The transfersomes furthermore can contribute to stabilising hydrolysis-sensitive materials and to make an improved distribution of agents in the sample and at the site of application possible, as well as to ensuring a more advantageous temporal course of the action of the active ingredient. The basic substance, of which the transfersomes consist, can itself have an advantageous effect. The most important carrier property, however, is to enable material to be transported into and through the permeability barrier.

Pursuant to the invention, the formulations described are optimised for topical application at or in the vicinity of permeability barriers. The application on the skin or on the plant cuticle ought to be particularly interesting. (However, they are also well suited for oral (p.o.) or parenteral (i.v., i.m. or i.p.) administration, particularly if the composition of the transfersome is selected so that losses at the site of administration are small.) Substances or components, which are decomposed preferentially at the site of application, taken up particularly readily or diluted, are particularly valuable in the last respect, depending on the intended use.

In the medical area, preferably up to 50, frequently up to 10, especially fewer than 2.5 or even fewer than 1mg of carrier substance are applied per cm^2 of skin surface; the optimum amount depends on the carrier composition, the depth or duration of action aimed for as well as on the site of application. In the agrotechnical area, the amounts applied typically are lower and frequently less than $0.1 g/m^2$.

In particular, the total content of amphiphilic substance to be applied on human or animal skin ranges from 0.01 to 40% by weight of the transfersome, preferably from 0.1 to 15% by weight and especially from 1 to 10% by weight.

For application on plants, the total content of amphiphilic substance ranges from 0.000001 to 10% by weight, preferably from 0.001 to 1% by weight and especially from 0.01 to 0.1% by weight.

Depending on the application aimed for, the formulations, pursuant to the invention, may also contain suitable solvents up to a concentration, which is determined by the respective physical (no solubilisation or no shift in the optimum worth mentioning), chemical (no effect on the stability), or biological or physiological (few undesirable side effects) compatibility.

Preferably, unsubstituted or substituted hydrocarbons, such as halogenated, aliphatic, cycloaliphatic, aromatic or aromatic aliphatic hydrocarbons, such as benzene, toluene, methylene chloride or chloroform, alcohols, such as methanol or ethanol, butanol, propanol, pentanol, hexanol or heptanol, dihydroxypropane, erythritol, low molecular weight alkane carboxylate esters, such as alkyl acetates, ethers, such as diethyl ether, dioxane or tetrahydrofuran, or mixtures of these solvents come into consideration.

Overviews of the lipids and phospholipids which, in addition to those named above, are suitable for use in the sense of this application, are contained in 'Form and Function of Phospholipids' (Ansell & Hawthorne & Dawson, publisher), 'An Introduction to the Chemistry and Biochemistry of Fatty Acids and Their Glycerides' by Gunstone and in other review works. The lipids and surfactants mentioned, as well as other boundary-active materials, which come into consideration, and their manufacture are known. A survey of the commercially obtainable polar lipids, as well as of the trademarks, under which they are sold by manufacturing companies, is given in the yearbook 'McCutcheon's Emulsifiers & Detergents', Manufacturing Confectioner Publishing Co. A topical list of the pharmaceutically acceptable active ingredients is given, for example, in the 'Deutschen Arzneibuch' (German Pharmacopoeia) (and the respective annual edition of the 'Rote Liste'), furthermore also in the British Pharmaceutical Codex, the European Pharmacopoeia, the Farmacopoeia Ufficiale della Republica Italiana, the Japanese Pharmacopoeia, the Dutch Pharmacopoeia, the Pharmacopoeia Helvetica, the Pharmacopee Francaise, The United States Pharmacopoeia, The United States NF, etc. A detailed list of enzymes, suitable pursuant to the invention, is contained in the volume 'Enzymes', 3rd Edition (M. Dixon and E.C. Webb, Academic, San Diego, 1979) and topical new developments can be found in the 'Methods in Enzymology' series. Sugar-recognising proteins, which are of interest in connection with this invention, are described in the book 'The Lectins: Properties, Functions and Applications in Biology and Medicine' (I.E. Liener, N. Sharon, I.T. Goldstein, Eds., Academic, Orlando, 1986) as well as in topical technical publications; agrotechnically interesting substances are listed in 'The Pesticide Manual' (C.R. Worthing, S.B. Walter, Eds., British Crop Protection Council, Worcestershire, England, 1986, for example, 8th edition) and in 'Wirkstoffe in Pflanzenschutz und Schadlings-bekampfung' (Active Ingredients in Plant Protection and Pest Control), published by the industrie-Verband Agrar (Frankfurt); commercially obtainable antibodies are listed in the 'Linscott's Directory' catalogue, the most important neuropeptides are listed in 'Brain Peptides' (D.T. Krieger, M.J. Brownstein, J.B. Martin, Eds., John Wiley, New York, 1983), corresponding supplemental volumes (such as 1987) and other technical publications.

Manufacturing techniques for liposomes, which are predominantly also suitable for the manufacture of transfersomes, are described in 'Liposome Technology' (Gregoriadis, Ed., CRC Press) or in older reference works, such as 'Liposomes in Immunobiology' (Tom & Six, Eds., Elsevier), in

'Liposomes in Biological Systems' (Gregoriadis & Allison, Eds., Willey), in 'Targeting of Drugs' (Gregoriadis & Senior & Trouet, Plenum), etc., as well as in the relevant patent literature.

The stability and permeation capability of transfersomes can be determined by filtration, if necessary under pressure, through a fine-pored filter or through otherwise controlled mechanical whirling up, shearing or comminuting.

The following examples illustrate the invention without limiting it. Temperatures are given in °C, carrier sizes in nanometers, pressures in pascals and other quantities in conventional SI units.

Unless otherwise stated, ratios and percentages are molar and the measurement temperature is about 21 °C.

Examples 1-4

Summary:

0-500mg	phosphatidyl choline from soybeans CMC = 10^{-7} M (approx. 98% PC = SPC)
0-500mg	distearoyl glycerophosphoethanolamine triazopolyethylene glycol (5000) CMC = 10^{-9} M
450mL	buffer, pH 7.3

Preparation:

Mixtures of SPC (molecular weight assumed to be 800Da) with increasing amounts of 0, 30 and 40 mole percent DSPE-PEG (molecular weight assumed to be 5800Da) and pure DSPE-PEG liposomes not containing any SPC are prepared. Subsequently, the mixtures obtained were dissolved in a chloroform/ methanol solution. After that, the lipid solution is transferred to a round-bottom flask.

After removal of the solvent in a rotary evaporator, a thin lipid film remains behind on the wall of the flask. This film is dried further under vacuum (10Pa), subsequently hydrated by addition of buffer and suspended by mechanical stirring. A cloudy suspension is obtained which, as a rule, is very viscous. The size of the particles in the resulting suspension is determined by means of dynamic light scattering as well as by means of microscopy. In all cases, the particle size observed is always greater than 0.5µm. Therefore, for the mixtures investigated, micelle formation and consequently also solubilisation can be excluded by means of dynamic light scattering.

The liposomes for the comparison experiments are prepared from a pure phosphatidyl choline by a similar method.

Determination of the Permeation Capability of the Carrier:

A carrier suspension is driven under an externally applied pressure through the constrictions in an artificial permeation barrier. The amount of material passing in unit time through the constriction, is determined volumetrically or gravimetrically. From the total area (application area of the material), the (driving) pressure, the time and the amount of penetrate, the permeation capability (P) of the suspension in the system investigated is calculated as follows:

$$P = \frac{\text{amount of permeate}}{\text{time x area x driving pressure}}$$

The measurement is repeated independently for several pressures. The relative dependence of the permeation capability, which is a measure of the carrier deformability, is calculated from the results of such measurements as a function of mechanical stress or pressure. The value for a

hydrated, 1% solution, containing pure SPC, is approximately >0.01μL/MPa/s/cm² at a pressure of 0.3MPa (see Figure 3).

The permeation capability measurement for such experimental series is carried out at 62ºC, so as to ensure that both lipids exist as liquid phases.

The results of such a series of measurements for Examples 1 to 4 are shown in Table 1. Table 1 shows that, as the driving pressure increases, the permeation capability greatly increases, but not linearly and, at high droplet loads (0.7MPa), is several orders of magnitude higher than the value, which results at a lower load (0.3MPa). Such a pronounced nonlinear relationship, however, arises exclusively (in the sense of a difference criterion) for transfersomes and not for liposomes. It is clearly evident from Figure 3 that the value of the permeation capability is several orders of magnitude lower for liposomes than tor transfersomes. This difference in permeation capability between transfersomes and liposomes clearly shows that the penetration capability for transfersomes is significantly increased over that for liposomes.

Table 1

Sample Final Size Description	Pressure(MPa)	Permeation Capability (μL/MPa/sec/cm²)	Initial Size	
			(nm)	(nm)
SPC/DSPE - PEG	0.7	22.3	225.7	92.5
70/3Omole%	0.6	18.7		94.5
10% lipid solution	0.5	10.9		96.1
rehydrated	0.4	2.8		96.1
sample	0.3	0.007		100.5
SPC/DSPE-PEG	0.7	12.2	217.3	96.3
60/40 mole %	0.6	13.2		100.7
10% Lipid solution	0.5	12.2		120
Rehydrated	0.4	3.39		99.1

Composition:

410.05 mg, 809.25 mg	phosphatidyl choline from soy beans (purer than 95%) CMC = 10^{-7} M
289.95 mg, 190.75 mg	didecanoyl phosphatidyl choline CMC ≈ 10^{-8}
7 mL, 10 mL	buffer, pH 7.3

Preparation:

The respective lipid content is selected so that both lipid components are present in a molar ratio of 1:1 or 3:1 in the final formulation. The appropriate amounts of phospholipid are weighed into a 50mL round-bottom flask and dissolved in each case in 1mL of 1:1 chloroform/methanol. After removal of the solvent in the rotary evaporator, a suspension of the film is obtained as described in Examples 1 to 4 and has carriers with an average radius of approximately 450nm.

Determination of the Carrier Permeation Capability

The carrier permeation capability is determined by the methods described in Examples 1 to 4. The corresponding results are shown in Figure 4. They indicate that an addition of didecanoyl phosphatidyl choline significantly increases the permeation capability of the carrier as a function of the concentration, particularly at high pressures. The carriers, formed from SPC and didecanoyl phosphatidyl choline in a molar ratio of 1:1 (with the exception of the carriers with a molar ratio of 3:1), have a significantly higher permeation capability than do the liposomes formed from pure SPC.

The values of the permeation capability for the carriers of Examples 5 to 6, which were measured, are summarised in Table 2.

The 10% suspension, containing the pure didecanoyl phosphatidyl choline, is milky cloudy. This suspension contains carriers with an average diameter of 700 ± 150nm and forms a sediment. This behaviour clearly shows that the lipid cannot be solubilised in the relevant concentration range either by itself or in combination with SPC.

Table 2

Sample Description	Pore Diameter (nm)	Pressure (MPa)	Permeation Capability
Sample: 3: 1	50	0.9	0.00039
	100	0.5	0.0083
		0.6	0.021
		0.7	0.04
		0.8	0.05
		0.9	0.066
Sample: 1: 1	50	0.9	0.16
	100	0.5	0.052
			0.021
		0.6	0.12
		0.7	0.17
			0.27
			0.22
		0.8	0.76
			0.69
		0.9	0.66
			0.60

Example 7

345.6mg	phosphatidyl choline from soy beans (purer than 95%, PC) CMC 10^{-7}M
154.4mg	distearoyl phosphomaltobionamide CMC= 10^{-8}M
4.5mL	buffer, pH7.3

A suspension of SPC/DSPE maltobionamide is prepared in a molar ratio of 3:1 according to the method described for Examples 5 to 6. The resulting carriers have an exceptionally good permeation capability. For the determination of the permeation capability, the size of the carrier is determined before and after each measurement. The measurements prove that there is no solubilisation of the carrier at any time.

The permeation capability of the carrier is determined at a pressure of 0.4MPa and in, contrast to Examples 5 to 6, at a temperature of 52°C. At this pressure, the permeation of the carrier through the artificial permeation barrier is observed to be adequately good. The lipid added (glycolipid) is incapable of solubilising the phospholipid. An investigation of the suspension by means of dynamic light scattering as well as by optical microscopy gives no indication of the existence of the solubilised (micellar) phase. The final size of the particles after permeation through the artificial permeability barrier depends on the driving pressure (0.3 to 0.9MPa; as the pressure increases, the tendency decreases) and is between 98 and 81nm.

Pure glycolipid does not dissolve or form a micellar suspension; instead, it forms a vesicle suspension. In order to prove this, an experiment was carried out, with which the osmotic activity of

DSPE in the aqueous medium can be determined. For this purpose, the lipid suspension was diluted with water. Because of the thereby arising concentration gradient, water enters the vesicles. As a direct consequence, the average vesicle radius increases measurably. On the other hand, particles without an internal volume (such as mixed micelles), do not change their size under comparable experimental conditions.

Examples 8-17

Composition:

203 - 86.5µL	phosphatidyl choline from soy beans (as a 1:1 weight/volume SPC solution in absolute ethanol) CMC (in water) ≈ 10-7 M
9.04-61.4 mg	diclofenac, solubility ≤ 10^{-3} M
1 mL	phosphate buffer (nominal): pH 6.5

The carriers are prepared as SPC/diclofenac mixtures in a molar ratio of 4: 1 to 1: 4 by the method described in Examples 1 to 4.

The mixtures so obtained are exposed to a source of ultrasound, until the samples are clear macroscopically (for approximately 4 minutes). After that, the solutions are centrifuged for 15 minutes at 15 000/rpm. The resulting 1:1 to 1:4 solutions are not clear (Figure 5); instead they are opalescent. On the other hand, the 4:1, 3:1 and 2:1 mixtures show clear deposits. After being allowed to stand for 5 minutes, the other suspensions also become cloudy, a flaky precipitate being formed by the 1:2, 1:3 and 1:4 mixtures (Table 3). The preparations show this behaviour even after the pH is adjusted with HCl to values between 7 and 7.2.

The carrier permeation capability, which is a measure of the carrier deformability, is determined as described in the preceding examples. For mixtures with 15mg/mL, 20mg/mL and 25mg/mL of diclofenac, at a pressure of 0.3MPa (driving pressure), the following permeability values (IP) are obtained: 6 x 10^{-11}m/Pa/s, 10^{-10}m/Pa/s and 2.5 x 10^{-10}m/Pa/s.

These values are comparable with those of known transfersomes, which were measured under similar conditions (SPC/NaChol 3/1 M/M; 2% by weight: 3 x 10^{-10}m/Pa/s). This proves that SPC/diclofenac mixtures of suitable composition have a very high permeation capability and consequently must be extremely deformable, although they cannot be solubilised at any time or any concentration.

Table 3

The pH is adjusted to a value between 7 and 7.2 with HCl and the mixture is ultrasonicated.

After ultrasonication:	1: 1.0	slightly cloudy
	1: 1.2	cloudy, liquid, crystals in solution about 20 per sight field
	1: 1.4	cloudy, liquid, crystals in solution about 20 per sight field
	1: 1.6	cloudy, liquid, crystals somewhat larger
	1: 1.8	cloudy, viscous, crystals ball together
	1: 2.0	cloudy, viscous, very many crystals
	1: 2.2	cloudy, viscous, very many very large crystals

Composition:

475 - 325mg	phosphatidyl choline from soy beans CMC ≈ 10^{-7}M
25 - 175mg	ibuprofen, solubility ≤ 5 x 10^{-5} M
5mL	buffer, pH 6.5

Preparation:

The preparation is as described in Examples 1 to 4, with the exception that, after the mixture is suspended, the pH is adjusted to a value of 7 by the addition of 10M NaOH. In each case, 5mL of ibuprofen-containing transfersomes are prepared with increasing amounts of ibuprofen and decreasing amounts of SPC (in 25mg steps), the total lipid concentration being 10%.

Microscopic Check of the Suspensions Obtained:

Sample 1: no crystals, very large carriers,
Sample 2: no crystals, very large carriers,
Sample 3: only flickering in background,
Sample 4: small crystals very occasionally,
Sample 5: no crystals, droplets,
Sample 6: predominantly crystals,
Sample 7: droplets, isolated very large crystals.

The determination of the carrier permeation capability is carried out as described in the preceding examples. The results of this measurement are shown in Figures 6 and 7. The mixtures of phospholipid and active ingredient investigated show typical transfersome behaviour throughout, but particularly in the concentration range of 35mg of ibuprofen per mL and above. The ibuprofen concentration of the carriers brings about no solubilisation.

Comparison Examples A-E

Comparison Example A (Example 2 of EP-A 0211 647)

Composition:

120mg	dipalmitoyl phosphatidyl choline (DPPC)
24mg	oleic acid
20mg	arginine
60mL	PBS (dissolve one tablet in 200 mL of distilled water)

DPPC (120.0mg) and 24.1mg of oleic acid were weighed into a 100mL beaker. Subsequently, the two reagents were mixed. A phosphate buffer salt (PBS) tablet was dissolved completely in 200mL of distilled water in order to obtain a 10mM (PBS) buffer. Arginine (20mg) was then dissolved in 60mL of PBS with a pH of 7.46 and added to the lipid mixture. The solution obtained was heated for 30 minutes at 40° to 45°C and stirred.

Comparison Example B (Example 9 of EP-A 0211 647)

27 mg	dipalmitoyl phosphatidyl choline (DPPC)
30mg	DSPC
60 mg	1-octadecane sulfonic acid (ODS)

DPPC (270.05 mg), 30.1 mg of DSPC and 60.1mg of 1-octadecane sulfonic acid (ODS) were dissolved in 1:1 chloroform/methanol. The sample was evaporated to dryness for 2 hours in a rotary evaporator. Subsequently, drying was continued for a further hour under vacuum. The residue was rehydrated with 10mL of PBS. The mixture was heated to 60°C and homogenised. After that, the sample was exposed for 5 minutes to ultrasound.

Comparison Example C (Example 7 of WO 88/07832):
Composition:

400 mg	Setacin F special paste(disodium lauryl sulfosuccinate)
680 mg	hydrogenated PC (PHPC)
280 mg	Minoxidil
	acetate buffer pH 5.5

Setacin F special paste (400mg), 580.03mg of PHPC and 200.03mg of Minoxidil were weighed into a beaker and dissolved in 1:1 chloroform/methanol and transferred to a round-bottom flask. The lipid mixture was concentrated for about 2.5 hours in a rotary evaporator and subsequently dried completely under vacuum. The sample was then shaken in a warm water bath at 50°C and rehydrated with 10mL of acetate buffer. After the sample has gone into solution completely, the solution is allowed to stand for 1 hour in the water bath shaker.

As antioxidant, 1mg of deferoxamine-mesylate were added. The pH of the solution was then adjusted to a value of about 7.24 by the addition of 1 drop of 10mM HCl. The solution could be homogenised macroscopically by stirring at a water bath temperature of 35°C.

Comparison Example D (Example 4 of EP-A 0220 797)

Composition:

400mg	purified hydrogenated soybean lecithin
40mg	HCO-60 (ethoxylated hydrogenated castor oil)
100mg	vitamin E
9.46mL	doubly distilled water

Phospholipon 90H (hydrogenated soybean lecithin, 400.04mg), 40mg of Emulgin HRE 60 (ethoxylated hydrogenated castor oil) and 100.11mg of vitamin E were weighed into a 100mL beaker and 9.46mL of doubly distilled water were added. The sample was stirred for 45 minutes, until almost everything had dissolved. The lipid solution was then exposed for 10 minutes at 79°C to ultrasound. To complete the dissolving, the sample was stirred once again and exposed to ultrasound for 10 minutes at 56°C.

Comparison Example E (Example 2 of EP-A 0 102 324)

Composition:

300mg	SPC
150mg	octadecyltrimethylammonium bromide
2550µL	distilled water

SPC (300mg) and 150mg of octadecyltrimethylammonium bromide were weighed into a 100mL beaker and dissolved in 1mL of 1:1 chloroform/ methanol.

The sample was evaporated to dryness under vacuum. A 1% solution was prepared by the addition of distilled water. The solution obtained was stirred for 15 minutes.

Unless stated otherwise, samples of the Comparison Examples A to E were prepared in accordance with the directions given in the publications named.

In Figure 8, the permeation capability (at a constant pressure of 0.9MPa) is shown for the Comparison Examples A to E and for an inventive ibuprofen/SPC transfersome in the form of a bar graph. It is clearly evident from the bar graph (Figure 8) that, at an elevated pressure (0.9MPa), the permeation capability of the compositions of the Comparison Examples A to E is significantly less than that of the inventive transfersomes.

The claims defining the invention are as follows:

1. Preparations for the application, administration or transport of at least one active ingredient into and through barriers and constrictions in the form of liquid droplets, which can be suspended in a liquid medium and are provided with a membrane-like sheath of one or a few layers of amphiphilic carrier substance, the carrier substance comprising at least two physicochemically different components, characterised in that at least two components are provided, which differ in their solubility in the suspension medium of the preparations by a factor of at least 10 and in that the content of solubilising components is less than 0.1 mole percent, based on the content of these substances, at which the solubilisation point of the enveloped droplets is reached or this solubilisation point cannot be reached.

2. The preparation of claim 1, characterised in that the active ingredient is for medicinal or biological purposes.

3. The preparation of claim 1 or claim 2, characterised in that the barriers and constrictions are skin or the like.

4. The preparation of any one of claims 1 to 3, characterised in that the suspension medium of the preparations is water.

5. The preparation of any one of claims 1 to 4, characterised in that the amphiphilic components are selected so that, independent of concentration, there is no solubilisation.

6. The preparation of any one of claims 1 to 5, characterised in that the solubility, especially the water solubility of the more soluble component(s) is/are at least 10^{-3} to 10^{-6} M and the solubility, especially the water solubility, of the less soluble component(s) is/are at least 10^{-6} to 10^{-10} M.

7. The preparation of claim 6, characterised in that the solubility is water solubility.

8. The preparation of any one of claims 1 to 7, characterised in that the difference between the solubility of the more soluble component(s) and the less soluble component(s) is approximately between 10^{1} and 10^{7}.

9. The preparation of claim 8, characterised in that the difference between the solubility of the more soluble component(s) and the less soluble component(s) is approximately between 10^{2} and 10^{6}.

10. The preparation of claim 8, characterised in that the difference between the solubility of the more soluble component(s) and the less soluble component(s) is approximately between 10^{3} and 10^{5}.

11. The preparation of any one of the claims 1 to 10, characterised in that the ability of the preparation to permeate through constrictions is at least 0.001% of the permeability of small molecules, which permeate essentially without being impeded.

12. The preparation of claim 11, characterised in that the ability of the preparation to permeate through constrictions is at least 0.1%.

13. The preparation of any one of claims 1 to 12, characterised in that the ratio of the permeation capability relative to reference particles $P_{(tracer)}/P_{(ref.)}$, the reference particles being, much smaller than the constrictions in the barrier, when the barrier itself is the site of the determination, is between 10^{-3} and 1.

14. The preparation of claim 13, characterised in that the reference particles are water.

15. The preparation of claim 13 or claim 14, characterised in that the ratio of the permeation capability relative to reference particles $P_{(transfer)}/P_{(refer)}$, is between 10^{-4} and 1.

16. The preparation of claim 13 or claim 14, characterised in that the ratio of the permeation capability relative to reference particles $P_{(transfer)}/P_{(refer)}$, is between 10^{-2} and 1.

17. The preparation of any one of claims 1 to 16, characterised in that the preparations contain at least two amphiphilic components of different solubility, for forming a carrier substance and/or a membrane-like sheath about a droplet amount of hydrophilic liquid, wherein the active ingredient is contained in the carrier substance in or at the membrane-like sheath and/or in the hydrophilic liquid.

18. The preparation of any one of claims 1 to 17, characterised in that the vesicle radius of the enveloped droplets is between about 25 and about 500nm.

19. The preparation of claim 18, characterised in that the vesicle radius of the enveloped droplets is between about 50 and about 200nm.

20. The preparation of claim 18, characterised in that the vesicle radius of the enveloped droplets is between 80 and about 100nm.

21. The preparation of any one of claims 1 to 20, characterised in that the sheath is a double layer.

22. The preparation of any one of claims 1 to 21, characterised in that the amphiphilic component (n) comprises or comprise physiologically tolerated lipids of different polarity and/or such as active ingredient or ingredients.

23. The preparation of any one of claims 1 to 22, characterised in that the amphiphilic substance comprises a lipid or lipoid of biological origin or a corresponding synthetic lipid or a derivative of such lipids, which forms stable structures.

24. The preparation of claim 23, characterised in that the amphiphilic substance comprises diacyl or dialkyl glycerophosphoethanolamino azo polyoxyethylene derivative, didecanoyl phosphatidyl choline, diacyl phosphooligomaltobionamide, a glyceride, a glycerophospholipid, isoprenoid lipid, sphingolipid, steroid, sterol, a sulfur-containing or hydrocarbon-containing lipid or a different lipid.

25. The preparation of claim 23 or claim 24, characterised in that the stable structures are double layers.

26. The preparation of claim 24 or claim 25, characterised in that the lipid which forms stable structures comprises a half protonated liquid fatty acid.

27. The preparation of claim 26, characterised in that the half protonated liquid fatty acid is a phosphatidyl choline, phosphatidyl ethanol amine, phosphatidyl glycerol, phosphatidyl inositol, a phosphatid acid, a phosphatidyl seine, a sphingomylein or sphingophospholipid, glycosphingolipid, ganglioside or other glycolipid, or a synthetic lipid, or a corresponding dialkyl or sphingosin derivative, glycolipid or other identical chain or mixed chain acyl lipid or alkyl lipid.

28. The preparation of claim 27, characterised in that the glycosphingolipid is cerebroside, ceramide polyhexoside, sulfatide or sphingoplasmalogen.

29. The preparation of claim 27, characterised in that the synthetic lipid is a dioleoyl, dilinolyl, dilinolenyl, dilinoloyl, dilinolinoyl or diarachinoyl, dilauroyl, dimyristoyl, dilalmitoyl, distearoyl phospholipid or a corresponding dialkyl or sphingosin derivative.

30. The preparation of any one of claims 1 to 29, characterised in that the less soluble amphiphilic component comprises a synthetic lipid, and the more soluble component or components is derived from one of the less soluble components listed above and, for increasing the solubility, is derivatised with a butanoyl, pentanoyl, hexanoyl, heptanoyl, octanoyl, nonanoyl, decanoyl, dodecane or undecanoyl or a corresponding monounsaturated or polyunsaturated or chain-branched substituent thereof or several substituents, selected independently of one another, and/or is substituted, complexed and/or associated with a different material, which is suitable for improving the solubility.

31. The preparation of claim 30, characterised in that the synthetic lipid is myristoleoyl, palmitoleoyl, petroselinyl, petroselaidyl, oleoyl, elaidyl, cis- or trans-vaccenoyl, linolyl, linolenyl, linolaidyl, octadecatetraenoyl, gondoyl, eicosaenoyl, eicosadienoyl, eicosatrienoyl, arachidoyl, cis- or trans-docosaenoyl, docosadienoyl, docosatrienoyl, dcocsatetraenoyl, caproyl, lauroyl, tridecanoyl, myristoyl, pentadecanoyl, palmitoyl, heptadecanoyl, stearoyl or nonadecanoyl, glycerophospholipid or a corresponding chain-branched derivative or a corresponding sphingosin derivative, glycolipid or a different acyl lipid or alkyl lipid;

32. The preparation of any one of claims 1 to 31, characterised in that the total content of amphiphilic substance for administration on human or animal skin is between 0.01 and 40% by weight of the preparation.

33. The preparation of claim 32, characterised in that the total content of amphiphilic substance for administration on human or animal skin is between 0.1 and 15% by weight.

34. The preparation of claim 32, characterised in that the total content of amphiphilic substance for administration on human or animal skin is between 1 and 10% by weight.

35. The preparation of any one of claims 1 to 34, characterised in that the total content of amphiphilic substance for application on plants is 0.000001 to 10% by weight.

36. The preparation of claim 35, characterised in that the total content of amphiphilic substance for application on plants is between 0.001 and 1% by weight.

37. The preparation of claim 35, characterised in that the total content of amphiphilic substance for application on plants is between 0.01 and 0.1% by weight.

38. The preparation of any one of claims 1 to 37, characterised in that, as active ingredient, it contains an adrenocorticostatic agent, a β-adrenolytic agent, an androgen or antiandrogen, an anti-parasitic, anabolic, anaesthetic or analgesic, analeptic, anti-allergic, anti-arrhythmic, anti-arteriosclerosis, anti-asthmatic and/or bronchospasmolytic agent, an antibiotic, an anti-depressive and/or anti-psychotic agent, an anti-diabetic agent, an antidote, an anti-emetic, anti-epileptic, anti-fibrinolytic, anti-convulsive or anti-cholinergic agent, an enzyme, coenzyme or a corresponding inhibitor, an antihistamine, an antihypertensive drug, a biological activity inhibitor, an antihypotensive agent, an anticoagulant, an anti-mycotic or antimyasthenic agent, an active ingredient against Parkinson's or Alzheimer's disease, an anti-phlogistic, anti-pyretic or anti-rheumatic agent, an antiseptic, a respiratory analeptic or stimulating agent, a broncholytic, cardiotonic or chemotherapeutic

agent, a coronary dilator, a cytostatic agent, a diuretic, a ganglion blocker, a glucocorticoid, a therapeutic agent for influenza, a haemostatic or hyptonic agent, immunoglobulin or fragment or a different immunological or receptor substance, a bioactive carbohydrate (derivative), a contraceptive, a migraine agent, a mineral corticoid, a morphine antagonist, a muscle relaxant, a narcotic, a neural or CNS therapeutic agent, a nucleotide or polynucleotide, a neuroleptic agent, a neuron transmitter or a corresponding antagonist, a peptide (derivative), an ophthalmic agent, a (para)-sympathicomimetic or (para)-sympathicolytic agent, a protein (derivative), a psoriasis/neurodermatitis agent, a mydriatic agent, a mood elevator, rhinological agent, a sleeping draft or its antagonist, a sedative, a spasmolytic, tuberculosis or urological agent, a vasoconstrictor or dilator, a virostatic agent or a wound-healing agent or several such agents.

39. The preparation of claim 38, characterised in that, as active ingredient, it contains diclofenac or ibuprofen.

40. The preparation of any one of claims 1 to 39, characterised in that the active ingredient is a nonsteroidal anti-inflammatory drug.

41. The preparation of claim 40, characterised in that the nonsteroidal anti-inflammatory drug is diclofenac, ibuprofen or a lithium, sodium, potassium, caesium, rubidium, ammonium, monoethyl, dimethyl, trimethylammonium or ethylammonium salt thereof.

42. The preparation of any one of claims 1 to 41, characterised in that the less polar component comprises a physiologically compatible lipid and the active ingredient is the more soluble component, optionally with the addition of less than 10% by weight, based on the total composition of the preparation, of a further soluble component, which is the more soluble component, and the total lipid concentration being between 0.005% by weight and 40% by weight and preferably between 0.5% by weight and 15% by weight and especially between 1% by weight and 10% by weight.

43. The preparation of claim 42, characterised in that the physiologically compatible lipid is from the class of phospholipids.

44. The preparation of claim 42, characterised in that the physiologically compatible lipid is from the class of phosphatidyl cholines.

45. The preparation of any one of claims 42 to 44, characterised in that the concentration of the more soluble component(s) is between 0.01% by weight and 15% by weight.

46. The preparation of any one of claims 42 to 44, characterised in that the concentration of the more soluble component(s) is between 0.1% by weight and 10% by weight.

47. The preparation of any one of claims 42 to 44, characterised in that the concentration of the more soluble component(s) is between 0.5% by weight and 3% by weight.

48. The preparation of any one of claims 42 to 47, characterised in that the total lipid concentration is between 0.5% by weight and 15% by weight.

49. The preparation of any one of claims 42 to 47, characterised in that the total lipid concentration is between 1% by weight and 10% by weight..

50. The preparation of any one of claims 1 to 49, characterised in that the preparation comprises consistency modifiers, antioxidants and/or stabilisers.

51. The preparation of claim 50, characterised in that the consistency modifiers are hydrogels.

52. The preparation of claim 50, characterised in that the antioxidants are probucol, tocopherol, BHT, ascorbic acid or desferroxamine.

53. The preparation of claim 50, characterised in that the stabilisers are phenol, cresol or benzyl alcohol.

54. The preparation of any one of claims 1 to 53, characterised in that the active ingredient is a growth regulating substance for living beings.

55. The preparation of any one of claims 1 to 54, characterised in that the active ingredient has biocidal properties.

56. The preparation of claim 55, characterised in that the active ingredient is an insecticide, pesticide, herbicide or fungicide.

57. The preparation of any one of claims 1 to 53, characterised in that the active ingredient is an allurement, in particular, a pheromone.

58. The preparation of claim 57, characterised in that the allurement is a pheromone.

59. Preparations for the application, administration or transport of at least one active ingredient into and through barriers and constrictions in the form of liquid droplets, which can be suspended in a liquid medium and are provided with a membrane-like sheath of one or a few layers of amphiphilic carrier substance, the carrier substance comprising at least two physicochemically different components, substantially as hereinbefore described with reference to any one of the examples.

60. A method for producing a preparation for the administration, application or transport of at least one active ingredient into and through natural barriers and constrictions, in the form of liquid droplets, which can be suspended in a liquid medium and are provided with a membrane-like sheath of one or a few layers of amphiphilic carrier substance, the carrier substance comprising at least two physicochemically different components, characterised in that at least two amphiphilic components are selected, which differ in their solubility in the suspension medium of the preparation, by a factor of at least 10 and the content of solubilising components is less than 0.1 mole percent, based on the content of these substances, at which the solubilising point of the enveloped droplets is reached or this point cannot be reached in a practically relevant region, and the content of amphiphilic components is adjusted, so that the ability of the preparation to permeate through constructions is at least 0.001% of the permeability of small molecules.

61. The method of claim 60, characterised in that the active ingredient is for medicinal or biological purposes.

62. The method of claim 60 or claim 61, characterised in that the barriers and constrictions are skin or the like.

63. The method of any one of claims 60 to 62, characterised in that the suspension medium of the preparations is water.

64. The method of any one of claims 60 to 63, characterised in that the small molecule is water.

65. The method of any one of claims 60 to 64, characterised in that the content of amphiphilic components is adjusted, so that the ratio of the permeation capability relative to reference particles, which are much smaller than the constrictions in the barrier when the barrier itself is the site of determination, is between 10^{-5} and 1.

66. The method of claim 65, characterised in that the barrier is water.

67. The method of claim 65 or claim 66, characterised in that the ratio of the permeation capability relative to reference particles is between 10^{-4} and 1.

68. The method of claim 65 or claim 66, characterised in that the ratio of the permeation capability relative to reference particles is between 10^{-2} and 1.

69. The method of any one of claims 60 to 68, characterised in that stability and permeation capability are determined by filtration, optionally under pressure, through a fine-pored filter or through otherwise controlled mechanical whirling up, shearing or comminuting.

70. The method of any one of claims 60 to 69, characterised in that the substance mixture for producing a transfersome-like preparation is subjected to a filtration, to a treatment with ultrasound, to stirring, to shaking or to other mechanical comminuting effects.

71. The method of any one of claims 60 to 70, characterised in that transfersome-like droplets, which form the preparation, are produced from at least two amphiphilic components of different polarity, at least one polar liquid and at least one active ingredient.

72. The method of any one of claims 60 to 70, characterised in that the transfersome-like droplets, which form the preparation, wherein the amphiphilic component(s) comprises or contains the active ingredient, are formed from at least two amphiphilic components of different polarity and at least one polar liquid.

73. The method of any one of claims 60 to 70, characterised in that the amphiphilic components and the hydrophilic substance in each case are mixed separately with the active ingredient and optionally brought into solution, the mixtures or solutions are then combined into a mixture, in which droplet formation is brought about by supplying energy.

74. The method of claim 73, characterised in that the energy is mechanical energy.

75. The method of any one of claims 60 to 74, characterised in that the amphiphilic components, either as such or dissolved in a physiologically compatible solvent or dissolving intermediary, which is miscible with a polar liquid or liquids, especially with water, are combined with a polar solution.

76. The method of any one of claims 60 to 75, characterised in that the formation of enveloped droplets is brought about by stirring, by evaporation from a reverse phase, by an injection method or a dialysis method, by electrical, thermal or mechanical stressing.

77. The method of claim 76, characterised in that the electrical, thermal or mechanical stressing is shaking, stirring, homogenising, ultrasonicating, rubbing, freezing or thawing, heating or cooling or high pressure or low pressure filtration.

78. The method of any one of claims 60 to 77, characterised in that the formation of the enveloped droplets is brought about by filtration and the filter material has a pore size of 0.01 to 0.8µm, several filters optionally being connected in series.

79. The method of claim 78, characterised in that the pore size is 0.05 to 0.3µm.

80. The method of claim 78, characterised in that the pore size is 0.08 to 0.15µm.

81. The method of any one of claims 60 to 80, characterised in that the association between carrier and active ingredients takes place at least partially after the droplet formation.

82. The method of any one of claims 60 to 81, characterised in that, shortly before use, the enveloped droplets are prepared from a concentrate or lyophilisate.

83. A method for producing a preparation for the administration, application or transport of at least one active ingredient into and through natural barriers and constrictions, in the form of liquid droplets, which can be suspended in a liquid medium and are provided with a membrane-like sheath of one or a few layers of amphiphilic carrier substance, the carrier substance comprising at least two physicochemically different components, substantially as hereinbefore described with reference to any one of the examples.

84. A preparation produced by the method of any one of claims 60 to 83.

Dated 4 July 2000
Idea Innovative Dermale Applikationen GmbH

Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

(12) AUSTRALIAN PATENT ABSTRACT
(19) AU (11) AU-A-17402/83

(54) LIPIDS AND TENSIDS IN AQUEOUS PHASE

(71) CIBA-GEIGY AG

(21) 17402/83 (22) 28.7.83 (24) 29.7.82
(31) 4597/82 (32) 29.7.82 (33) CH
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(74) WM

(57)

There is great interest in the therapeutic use of liposomes as carriers for a very wide range of active ingredients. Accordingly, liposomes have been proposed as carriers for proteins, e.g. antibodies or enzymes, hormones, vitamins or genes or, for analytical purposes, as carriers for marker compounds. For example, US patent 3 933 754 describes a chemotherapeutic process for treating tumour cells, wherein liposomes are used as drug carriers.

In the process of this invention it is possible to prepare, in simple manner and without using complicated apparatus, aqueous phases which contain small unilamellar liposomes (SUL) with a diameter of about 200 to 600 Å, and large unilamellar liposomes (LUL) with a diameter of about 600 to 3000 Å. Small unilamellar liposomes can be separated from large unilamellar liposomes by means of suitable separating methods, e.g. by gel filtration or in an ultrafiltration cell.

Claim

1. A process for the preparation of unilamellar liposomes in aqueous phase, which comprises dispersing a homogeneous mixture of an anionic surfactant and a lipid, in aqueous phase, at a concentration lower than the critical micelle concentration (cmc) of the surfactant in the

.../2

particular phase and, if necessary, neutralising the aqueous phase so obtained and, if desired, enriching and/or separating the resultant unilamellar liposomes.

2. A process according to claim 1, which comprises dispersing a homogeneous mixture of an anionic or cationic surfactant.

17402/83

COMMONWEALTH OF AUSTRALIA

Patents Act 1952-1969

CONVENTION APPLICATION FOR A PATENT

(1) Here insert the full Name or Names of Applicant or Applicants, followed by Address (es).

I/We (1) CIBA-GEIGY AG,

of Klybeckstrasse 141, 4002 Basle, Switzerland

(2) Here insert Title of Invention.

hereby apply for the grant of a Patent for an invention entitled (2)

LIPIDS AND TENSIDS IN AQUEOUS PHASE

(3) Here insert number(s) of basic application(s)

which is described in the accompanying complete specification. This application is a Convention application and is based on the application numbered (3)

4597/82-1

(4) Here insert Name of basic Country or Countries, and basic date or dates.

for a patent or similar protection made in (4) Switzerland

on 29th July 1982

~~My~~ Our address for service is Messrs. Edwd. Waters & Sons, Patent Attorneys, 50 Queen Street, Melbourne, Victoria, Australia.

DATED this 27th day of July 1983.

(5) Signature(s) of Applicant(s) or Seal of Company and Signature(s) of the Officers authorised by its Articles of Association

CIBA-GEIGY AG

by

W. F. Dancer

Reg'd. Patent Attorney

To:

AU Case 4-14035/+ FORM 8

COMMONWEALTH OF AUSTRALIA

17402/83 Patents Act 1952 - 1969

DECLARATION IN SUPPORT OF A CONVENTION APPLICATION FOR A PATENT

In support of the Convention Application made by CIBA-GEIGY AG for a patent for an invention entitled:

Lipids and tensids in aqueous phase

We, Arnold Seiler and Ernst Altherr) of CIBA-GEIGY AG, Klybeckstrasse 141, 4002 Basle, Switzerland

do solemnly and sincerely declare as follows:

1. We are authorised by the applicant for the patent to make this declaration on its behalf.

2. The basic application(s) as defined by Section 141 on the Act was made in Switzerland on July 29, 1982

by CIBA-GEIGY AG, 4002 Basle, Switzerland.

3. Helmut Hauser, Schwarzbachstrasse 91, 8713 Uerikon, Switzerland

is the actual inventor of the invention and the facts upon which the applicant is entitled to make the application are as follows: The said applicant is the assignee of the actual inventor.

4. The basic application referred to in paragraph 2 of this Declaration was the first application made in a Convention country in respect of the invention the subject of the application.

DECLARED at Basle, Switzerland on July 11, 1983

CIBA-GEIGY AG

pp

To: The Commissioner of Patents:

Form 10

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952-69

COMPLETE SPECIFICATION

(ORIGINAL)

Class Int. Class

Application Number: 17403/83
Lodged:

Complete Specification Lodged:
Accepted:
Published:

Priority :

Related Art :

Name of Applicant : CIBA-GEIGY AG

Address of Applicant : Klybeckstrasse 141, 4002 Basle, Switzerland

Actual Inventor: HELMUT HAUSER

Address for Service : EDWD. WATERS & SONS,
50 QUEEN STREET, MELBOURNE, AUSTRALIA, 3000.

Complete Specification for the invention entitled:

LIPIDS AND TENSIDS IN AQUEOUS PHASE

The following statement is a full description of this invention, including the best method of performing it known to :- US

Case 4-14035/+

Lipids and tensids in aqueous phase

The present invention relates to a process for the preparation of unilamellar liposomes in aqueous phase.

Liposomes have been described in the literature in a wide range of publications, and many investigations are concerned with their structure and use. A distinction is made between unilamellar liposomes having a double layer of lipids and multilamellar liposomes having a number of double layers of lipids of onion-like structure.

Unilamellar liposomers have a spherical shell and a diameter of about 200 to 50,000 Å, preferably of about 200 to 30,000 Å. The spherical shell consists of a double layer of the lipid components, e.g. amphiphatic lipids such as phospholipids, e.g. phosphatidic acid, lecithin or cephalin, with or without neutral lipids, e.g. cholesterol. This double layer surrounds a cavity which contains an aqueous phase.

There is great interest in the therapeutic use of liposomes as carriers for a very wide range of active ingredients. Accordingly, liposomes have been proposed as carriers for proteins, e.g. antibodies or enzymes, hormones, vitamins or genes or, for analytical purposes, as carriers for marker compounds. For example, US patent 3 933 754 describes a chemotherapeutic process for treating tumour cells, wherein liposomes are used as drug carriers.

The drug is encapsulated either during the formation of the liposomes or subsequently by diffusion. The preparation of liposomes and the encapsulation of the drug can be effected by different methods, a survey of which may be found in the article "Liposomes - Problems

and promise as selective drug carriers" by Stanley B. Kaye, Cancer Treatment Reviews (1981), 8, pp. 27-50. Further methods of preparing liposomes for encapsulating drugs are also described by Barenholz et al. in Biochemistry, Vol. 16, No. 12, 2806-2810, and also in German Offenlegungsschrift specifications 28 19 855, 29 02 672, 25 32 317 and 28 42 608, in US patent 4 053 585, and in European patent application 36 676.

In the prior art methods, the lipid components, e.g. phospholipids such as phosphatidic acid, lecithin or cephalin, with or without neutral lipids, e.g. cholesterol, are dissolved in an organic solvent, e.g. chloroform or benzene. After stripping off the solvent, there remains a homogeneous layer, e.g. a film, of the particular lipid components. The lipid components are subsequently dispersed in an aqueous phase which contains the appropriate drug, e.g. by shaking. Unilamellar liposomes which encapsulate the drug are formed in the course of the subsequent treatment with ultrasonic irradiation.

In the process of this invention it is possible to prepare, in simple manner and without using complicated apparatus, aqueous phases which contain small unilamellar liposomes (SUL) with a diameter of about 200 to 600 Å, and large unilamellar liposomes (LUL) with a diameter of about 600 to 3000 Å. Small unilamellar liposomes can be separated from large unilamellar liposomes by means of suitable separating methods, e.g. by gel filtration or in an ultrafiltration cell.

The present invention relates to a process for the preparation of unilamellar liposomes, which comprises dispersing a homogeneous mixture of an ionic surfactant and a lipid, in aqueous phase, at a concentration lower than the critical micelle concentration (cmc) of the surfactant in the particular phase and, if necessary, neutralising the aqueous phase so obtained and, if desired, enriching and/or separating the resultant unilamellar lipoeomes.

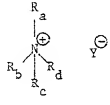
Throughout this specification, the general terms employed preferably have the meanings set forth below.

The term "lower" used to qualify organic radicals, e.g. lower alkyl, lower alkylene, lower alkoxy, lower alkanoyl etc., denotes that such radicals, unless otherwise expressly defined, contain from 1 to 7, preferably 1 to 4, carbon atoms.

The preparation of the homogeneous mixture of an ionic surfactant and a lipid is effected in a manner which is known per se and is described in the section entitled "Preparation of the homogeneous layer of the lipid components."

An ionic surfactant will be understood as meaning a cationic or anionic surfactant.

A cationic surfactant is e.g. a compound of the formula



(IA),

wherein R_a is an unsubstituted or substituted hydrocarbon radical, R_b is lower alkyl, phenyl-lower alkyl or hydroxy, R_c and R_d are lower alkyl, or R_b and R_c, together with the nitrogen atom to which they are attached, form an aliphatic heterocyclic ring system which may be substituted at a carbon atom, and R_d is lower alkyl, or R_b, R_c and R_d, together with the nitrogen atom to which they are attached, form an aromatic heterocyclic ring system, and $Y^{\ominus}$ is an anion.

In a cationic surfactant of the formula (IA), an unsubstituted or substituted aliphatic hydrocarbon radical R_a is for example lower alkyl substituted by aryloxy-lower alkoxy, or is straight chain or

branched alkyl containing 7 to 22, preferably 12 to 20, carbon atoms, or alkenyl containing 8 to 20, preferably 12 to 20, carbon atoms and 1 to 4 double bonds.

Aryl in arlyoxy-lower alkoxy is for example phenyl which may be mono- or disubstituted by straight chain C_1-C_4alkyl, e.g. methyl, ethyl or n-propyl, or by branched C_3-C_{10}alkyl, e.g. isobutyl, tert-butyl, amyl, neopentyl, 2- or 3-methylpentyl, 2,2- or 2,3-dimethylbutyl, 2- or 3-methylhexyl, 3-ethylpentyl, 2,2-, 2,3-, 2,4- or 3,3-dimethylpentyl, 4-methylheptyl, 2,2,2-, 2,2,4-, 2,3,3- or 2,3,4-trimethylpentyl, 1,1,3,3-tetramethylbutyl or 2,2,3,3-tetramethylbutyl.

Lower alkoxy in aryloxy-lower alkoxy is for example methoxy, ethoxy, n-propoxy or n-butoxy.

R_a as lower alkyl substituted by aryloxy-lower alkoxy is for example aryloxy-lower alkoxymethyl or 2-aryloxy-lower alkoxyethyl, e.g. aryloxy-methoxymethyl, 2-aryloxymethoxyethyl, 2-aryloxyethoxymethyl or 2-(2-aryloxyethoxy)ethyl, e.g. phenoxymethoxymethyl, 2-phenoxymethoxyethyl, 2-phenoxyethoxymethyl, 2-(2-phenoxyethoxy)ethyl, 2-, 3- or 4-methylphenoxymethyl, 2-(2-methylphenoxymethoxy)ethyl, 2-(3-methylphenoxymethoxy)ethyl, 2-(4-methylphenoxymethoxy)ethyl, 2-(2-methylphenoxy)ethoxymethyl, 2-(3-methylphenoxy)ethoxymethyl, 2-(4-methylphenoxy)ethoxymethyl, 2-[2-(2-methylphenoxy)ethoxy]ethyl, 2-[2-(3-methylphenoxy)ethoxy]ethyl, 2-[2-(4-methylphenoxy)ethoxy]ethyl, 4-(1,1,3,3-tetramethylbutyl)phenoxymethoxymethyl, 2-[4-(1,1,3,3-tetramethylbutyl)phenoxymethoxy]ethyl, 2-[4-(1,1,3,3-tetramethylbutyl)-phenoxy]ethoxymethyl, 2-[2-(4-(1,1,3,3-tetramethylbutyl)phenoxy)-ethoxy]ethyl, 2-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxymethoxyethyl, 2-[2-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxymethoxy]ethyl, 2-[3-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxymethoxy]ethyl, 2-[2-(2-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxy)]ethoxymethyl, 2-[2-(3-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxymethyl, 2-[2-(2-methyl-4-(1,1,3,3-tetramethylbutyl)-phenoxy)ethoxy]ethyl or 2-[2-(3-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxy)ethoxy]ethyl.

R_a as lower alkyl substituted by aryloxy-lower alkoxy is preferably 2-[2-(2-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxy)ethoxy]ethyl and 2-[2-(3-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxy)ethoxy]ethyl.

R_a as straight chain or branched alkyl of 7 to 22, preferably 12 to 20, carbon atoms, is for example n-heptyl, 2-methylhexyl, 3-methylhexyl, 3-ethylpentyl, 2,2-, 2,3-, 2,4- or 3,3-dimethylpentyl, n-octyl, 4-methylheptyl, 2,2,3-, 2,2,4-, 2,3,3-, 2,3,4-trimethylpentyl, n-nonyl, n-decyl, n-undecyl, n-dodecyl (lauryl), n-tridecyl, n-tetradecyl (myristyl), n-pentadecyl, n-hexadecyl (cetyl), n-heptadecyl, n-octadeyl (stearyl), n-nonadecyl or n-eicosyl (arachinyl).

Straight chain alkyl containing an even number of 12 to 20 carbon atoms is preferred, e.g. n-dodecyl (lauryl, n-tetradecyl (myristyl), n-hexadecyl (cetyl), n-octadecyl (stearyl) or n-eicosyl (arachinyl).

R_a as alkenyl containing 8 to 20, preferably 12 to 20, carbon atoms and 1 to 4 double bonds is for example octen-1-yl, nonen-1-yl, decen-1-yl, undecen-1-yl, dodecen-1-yl, 9-cis-dodecenyl (lauroleyl), tridecen-1-yl, tetradecen-1-yl, 9-cis-tetradecenyl (myristoleyl), pentadecen-1-yl, hexadecen-1-yl, 9-cis-hexadecenyl (palmitoleinyl), heptadecen-1-yl, octadecen-1-yl, 6-cis-octadecenyl (petroselinyl), 6-trans-octadecenyl (petroselaidinyl), 9-cis-octadecenyl (oleyl), 9-trans-octadecenyl (elaidinyl), 9-cis-12-trans-octadecadienyl (linoleyl), 9-cis-11-trans-13-trans-octadecatrienyl (α-eleostearinyl), 9-cis-11-trans-13-trans-octadecatrienyl (β-eleostearinyl), 9-cis-12-cis-15-cis-octadecatrienyl (linolenyl), 9-, 11-, 13-, 15-octadecatetraenyl (parinaryl), nonadecen-1-yl, eicosen-1-yl, 9-cis-eicosenyl (gadoleinyl), 5-, 11-, 14-eicosatrienyl or 5-, 8-, 11-, 14-eicosatetraenyl (arachidonyl).

Alkenyl containing 12 to 20 carbon atoms and one double bond is preferred, e.g. 9-cis-dodecenyl (lauroleyl), 9-cis-tetradecenyl (myristoleyl), 9-cis-hexadecenyl (palmitoleinyl), 6-cis-octadecenyl (petroselinyl), 6-trans-octadecenyl (petroselaidinyl), 9-cis-octadecenyl (oleyl), 9-trans-octadecenyl (elaidinyl) or 9-cis-eicosenyl (gadoleinyl).

R_b, R_c or R_d as lower alkyl is for example methyl or ethyl. R_b as phenyl-lower alkyl is for example benzyl or 2-phenylethyl.

An aliphatic heterocyclic ring system formed by R_b and R_c together with the nitrogen atom to which they are attached is for example a monocyclic 5- or 6-membered azacyclyl, oxaazacyclyl or thiazacyclyl radical, e.g. piperidino, morpholino or thiamorpholino. Substituents of this heteroring are the substituents R_a and R_d at the nitrogen and lower alkyl, e.g. methyl, ethyl, n-propyl or n-butyl, at a carbon atom.

A heterocyclic ring system formed by R_b and R_c together with the nitrogen atom and substituted at a carbon atom by lower alkyl is e.g. 2-, 3- or 4-methylpiperidinio, 2-, 3- or 4-ethylpiperidinio or 2- or 3-methylmorpholinio.

An aromatic heterocyclic ring system formed by R_b, R_c and R_d together with the nitrogen atom is e.g. a monocyclic 5- or 6-membered azacyclyl, diazacyclyl, oxaazacyclyl or thiazacyclyl radical, e.g. pyridinio, imidazolinio, oxazolinio or thiazolinio, or for example a monoaza-bicyclyl radical which is fused to a benzene ring, e.g. quinolinio or isoquinolinio. Substituents of this hetero-ring are the radical R_a at the nitrogen and lower alkyl at a carbon atom, e.g. methyl or ethyl, hydroxy-lower alkyl, e.g. hydroxymethyl or 2-hydroxyethyl, oxo, hydroxy or halogen, e.g. chlorine or bromine.

A heterocyclic ring system formed by R_b, R_c and R_d and substituted at a carbon atom by the above mentioned radicals is for example 2- or 4-lower alkylpyridinio, e.g. 2- or 4-methylpyridinio or 2- or 4-ethylpyridinio, di-lower alkylpyridinio, e.g. 2,6-dimethylpyridinio, 2-methyl-3-ethylpyridinio, 2-methyl-4-ethylpyridinio, 2-methyl-5-ethylpyridinio or 2-methyl-6-ethylpyridinio, 2-, 3- or 4-halopyridinio, e.g. 2-, 3- or 4-chloropyridinio or 2, 3- or 4-bromopyridinio, 2-lower alkylimidazolinio, 2-lower alkyloxazolinio or 2-lower alkylthiazolinio, e.g. 2-methylimidazolinio or 2-ethylimidazolinio, 2-ethyl- or 2-methyloxazolinio, 2-methyl- or 2-ethylthiazolinio or 2-lower alkyl-8-haloquinolinio, e.g. 2-methyl-8-chloroquinolinio.

An anion $Y^{\ominus}$ is for example a halide ion, e.g. the fluoride, chloride or bromide ion, a lower alkanoate, e.g. the formate or acetate ion, the hydrogen sulfate ion, a lower alkylsulfate ion, e.g. the methyl or ethyl sulfate ion, a lower alkylsulfonate ion, e.g. the methyl sulfonate ion, or an arylsulfonate ion, e.g. the phenyl sulfonate ion or the toluene sulfonate ion. An anion $Y^{\ominus}$ is preferably a halide ion, e.g. the chloride or bromide ion.

A cationic surfactant of the formula IA is preferably N-benzyl N,N-dimethyl-N-2-[2-(4-(1,1,3,3-tetramethylbutyl)phenoxy)ethoxy]ethyl-ammonium chloride, N-benzyl N,N-dimethyl-N-2-[2-(3-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxy)ethoxy]ethylammonium chloride (methyl-benzethonium chloride), n-dodecyltrimethylammonium chloride or bromide, trimethyl n-tetradecylammonium chloride or bromide, n-hexadecyl-trimethylammonium chloride or bromide (cetyltrimethylammonium chloride or bromide), trimethyl n-octadecylammonium chloride or bromide, ethyl n-dodecyldimethylammonium chloride or bromide, ethyldimethyl n-tetradecylammonium chloride or bromide, ethyl n-hexadecyldimethylammonium chloride or bromide, ethyldimethyl n-octadecylammonium chloride or bromide, n-alkyl benzyldimethylammonium chloride or bromide (benzalkonium chloride or bromide), e.g. benzyl n-dodecyldimethylammonium chloride or bromide, benzyl n-hexadecyldimethylammonium chloride or

bromide or benzyldimethyl n-octadecylammonium chloride or bromide, N-(n-decyl)pyridinium chloride or bromide, N-(n-dodecyl)pyridinium chloride or bromide, N-(n-tetradecyl)pyridinium chloride or bromide, N-(n-hexadecyl)pyridinium chloride or bromide (cetylpyridinium chloride or bromide), or N-(n-octadecyl)pyridinium chloride or bromide, or a mixture of these surfactants.

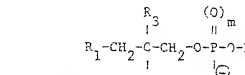
An anionic surfactant is for example

a) a compound of the formula

$$[R_a-(O-A)_m-B]^{\ominus}Z^{\oplus} \qquad \text{(IB)}$$

wherein R_a is an unsubstituted or substituted hydrocarbon radical, A is lower alkylene, m is 0 (direct bond) or 1, B is the sulfonate or sulfate group and $Z^{\oplus}$ is a monovalent cation, or

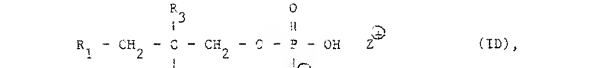
b) a compound of the formula



$$\text{(IC)},$$

wherein m is 0 or 1, one of R_1 and R_2 is hydrogen, hydroxy or lower C_1-C_4alkyl, and the other is alkyl, alkenyl, alkoxy, alkenyloxy or acyloxy, each of 10 to 20 carbon atoms, R_3 is hydrogen or lower C_1-C_4alkyl, and R_4 is unsubstituted or substituted lower C_1-C_7alkyl, a carbohydrate radical of 5 to 12 carbon atoms or, if both R_1 and R_2 are hydrogen or hydroxy, is a steroid radical, and $Z^{\oplus}$ is a monovalent cation, or

c) a compound of the formula



(ID),

wherein R_1, R_2, R_3 and $Z^{\oplus}$ are as defined for formula (IC).

In an anionic surfactant of the formula (IB), the unsubstituted or substituted hydrocarbon radical R_a is as defined for formula (IA) and is preferably straight chain or branched alkyl containing 7 to 22, preferably 12 to 20, carbon atoms, and alkenyl containing 6 to 20, preferably 12 to 20, carbon atoms and 1 to 4 double bonds.

In an anionic surfactant of the formula (IB), R_a is preferably straight chain alkyl containing an even number of 12 to 20 carbon atoms, for example n-dodecyl (lauryl), n-tetradecyl (myristyl), n-hexadecyl (cetyl), n-octadecyl (stearyl) or n-eicosyl (arachinyl), or is alkenyl containing 12 to 20 carbon atoms and one double bond, for example 9-cis-dodecenyl (lauroleyl), 9-cis-tetradecenyl (myristoleyl), 9-cis-hexadecenyl (palmitoleinyl), 6-cis-octadecenyl (petroselinyl), 6-trans-octadecenyl (petroselaidinyl), 9-cis-octadecenyl (oleyl), 9-trans-octadecenyl (elaidinyl) or 9-cis-eicosenyl (gadoleinyl).

A as lower alkylene is for example methylene, ethylene, n-propylene or n-butylene.

The cation $Z^{\oplus}$ is an alkali metal cation, e.g. the lithium, sodium or potassium cation, or is a tetra-lower alkylammonium cation, e.g. tetramethylammonium.

An anionic surfactant of the formula IB is preferably an alkali metal alkyl sulfate (m = 0), e.g. sodium or potassium n-dodecyl (lauryl) sulfate, sodium or potassium n-tetradecyl (myristyl) sulfate, sodium

or potassium n-hexadecyl (cetyl) sulfate or sodium or potassium n-octadecyl (stearyl) sulfate, an alkali metal alkyl ether sulfate (m = 1), e.g. sodium or potassium n-dodecyloxyethyl sulfate, sodium or potassium n-tetradecyloxyethyl sulfate, sodium or potassium n-hexadecyloxyethyl sulfate or sodium or potassium n-octadecyloxyethyl sulfate, or an alkali metal alkane sulfonate, e.g. sodium or potassium n-dodecane sulfonate, sodium or potassium n-tetradecane sulfonate, sodium or potassium n-hexadecane sulfonate or sodium or potassium n-octadecane sulfonate.

In an anionic surfactant of the formula IC, R_1, R_2 or R_3 as lower C_1-C_4alkyl is preferably methyl, and also ethyl, n-propyl or n-butyl.

R_1 or R_2 as alkyl is preferably n-decyl, n-undecyl, n-dodecyl (lauryl), n-tridecyl, n-tetradecyl (myristyl), n-pentadecyl, n-hexadecyl (cetyl), n-octadecyl (stearyl) and n-eicosyl (arachinyl).

R_1 or R_2 as alkenyl is preferably 9-cis-dodecenyl (lauroleyl), 9-cis-tetradecenyl (myristoleyl), 9-cis-hexadecenyl (palmitoleinyl), 6-cis-octadecenyl (petroselinyl), 6-trans-octadecenyl (petroselaidinyl), 9-cis-octadecenyl (oleyl), 9-trans-octadecenyl (elaidinyl) or 9-cis-eicosenyl (gadoleinyl).

R_1 or R_2 as alkoxy is preferably n-decyloxy, n-dodecyloxy (lauryloxy), n-tetradecyloxy (myristyloxy), n-hexadecyloxy (cetyloxy), n-octadecyloxy (stearyloxy) or n-eicosyloxy (arachinyloxy).

R_1 or R_2 as alkenyloxy is preferably 9-cis-dodecenyloxy (lauroleyloxy), 9-cis-tetradecenyloxy (myristoleyloxy), 9-cis-hexadecenyloxy (palmitoleinyloxy), 6-cis-octadecenyloxy (petroselinyloxy), 6-trans-octadecenyloxy (petroselaidinyloxy), 9-cis-octadecenyloxy (oleyloxy), 9-trans-octadecenyloxy (elaidinyloxy) or 9-cis-eicosenyl (gadoleinyloxy).

R_1 or R_2 as acyloxy is e.g. alkanoyloxy or alkenoyloxy.

R_1 or R_2 as alkanoyloxy is preferably n-decanoyloxy, n-dodecanoyloxy (lauroyloxy), n-tetradecenoyloxy (myristoyloxy), n-hexadecanoyloxy (palmitoyloxy), n-octadecanoyloxy (stearoyloxy) or n-eicosoyloxy (arachinoyloxy).

R_1 or R_2 as alkenoyloxy is preferably 9-cis-dodecenyloxy (lauroleoyloxy), 9-cis-tetradecenoyloxy (myristoleoyloxy), 9-cis-hexadecenoyloxy (palmitoleinoyloxy), 6-cis-octadecenoyloxy (petroselinoyloxy), 6-trans-octadecenoyloxy (petroselaidinoyloxy), 9-cis-octadecenoyloxy (oleoyloxy), 9-trans-octadecenoyloxy (elaidinoyloxy) or 9-cis-eicosenoyl (gadoleinoyloxy).

R_4 as lower C_1-C_7alkyl is e.g. methyl, ethyl, isopropyl, n-propyl, isobutyl or n-butyl, and may be substituted by acidic groups, e.g. carboxyl or sulfo, by acidic and basic groups, e.g. carboxyl and amino, in which case the amino group is in the α-position relative to the carboxyl group, by free or etherified hydroxyl groups, where two etherified hydroxyl groups may be linked to each other through a divalent hydrocarbon radical, e.g. by methylene, ethylene, ethylidene, 1,2-propylene or 2,2-propylene, by halogen, e.g. chlorine or bromine, by lower alkoxycarbonyl, e.g. methoxycarbonyl or ethoxycarbonyl, or by lower alkanesulfonyl, e.g. methanesulfonyl.

R_4 as substituted C_1-C_7alkyl is preferably carboxy-lower alkyl, e.g. carboxymethyl, 2-carboxyethyl or 3-carboxy-n-propyl, α-amino-ω-carboxy-lower alkyl, e.g. 2-amino-2-carboxyethyl or 3-amino-3-carboxy-n-propyl, hydroxy-lower alkyl, e.g. 2-hydroxyethyl or 2,3-dihydroxypropyl, lower alkoxy-lower alkyl, e.g. methoxy, methyl or ethoxymethyl, 2-methoxyethyl or 3-methoxy-n-propyl, lower alkylenedioxy-lower alkyl, e.g. 2,3-ethylenedioxypropyl or 2,3-(2,2-propylene)dioxypropyl, or halo-lower alkyl, e.g. chloromethyl or bromomethyl, 2-chloroethyl or 2-bromoethyl, 2- or 3-chloro-n-propyl

or 2- or 3-bromo-n-propyl.

R_4 as a carbohydrate radical of 5 to 12 carbon atoms is e.g. a natural monosaccharide radical which is derived from a pentose or hexose in the form of an aldose or a ketose.

A pentose in the form of an aldose is e.g. D-ribose, D-arabinose, D-xylose or D-lyxose. A pentose in the form of a ketose is e.g. D-ribulose or D-xylulose. A hexose in the form of an aldose is e.g. D-allose, D-altrose, D-glucose, D-mannose, D-galactose or D-talose. A hexose in the form of a ketose is e.g. D-psicose, D-fructose, D-sorbose or D-tagatose.

A hexose is preferably in cyclic form, e.g. in the form of a pyranose (aldose), e.g. α- or β-D-glucopyranose, or a furanose, e.g. α- or β-D-fructose. The pyranosyl radical is preferably esterified with the phosphatidyl group through the hydroxy group in the 1- or 6-position, and the furanosyl radical is esterified with the phosphatidyl group through the hydroxyl group in the 1- or 5-position (m = 1).

A carbohydrate radical R_4 of 5 to 12 carbon atoms is also a natural disaccharide radical, e.g. a disaccharide radical which is formed from two hexoses by condensation of two aldoses, e.g. D-glucose or D-galactose, or of an aldose, e.g. D-glucose, with a ketose, e.g. fructose.

Disaccharides formed from two aldoses, e.g. lactose or maltose, are preferably esterified with the phosphatidyl group through the hydroxyl group which is in the 6-position of the particular pyranosyl radical. Disaccharides formed from an aldose and a ketose, e.g. saccharose, are preferably esterified with the phosphatidyl group through the hydroxyl group which is in the 6-position of the pyranosyl radical or in the 1-position of the furanosyl radical (m = 1).

A carbohydrate radical R_4 of 5 to 12 carbon atoms is further a derived mono- or disaccharide radical, wherein e.g. the aldehyde group and/or one or two terminal hydroxyl groups are oxidised to carboxyl groups, and is e.g. a D-gluconic, D-glucaric or D-glucoronic acid radical which is preferably in the form of a cyclic lactone radical. Likewise, the aldehyde or keto group of a derived mono- or disaccharide radical can be reduced to hydroxyl groups, e.g. inositol, sorbitol or D-mannitol, or hydroxyl groups can be replaced by hydrogen, e.g. desoxy sugar, e.g. 2-desoxy-D-ribose, L-rhamnose or L-fucose, or by amino groups, e.g. amino sugar, e.g. D-glucosamine or D-galactosamine.

A carbohydrate radical R_4 can also be a fission product formed by reacting one of the mono- or disaccharides mentioned above with a strong oxidising agent, e.g. periodic acid.

A steroid radical R_4 is e.g. a sterol radical which is esterified with the phosphatidyl group through the hydroxyl group which is in the 3-position of the steroid skeleton (m = 1).

A sterol radical is e.g. lanosterol, sitosterol, coprostanol, cholestanol, glycocholic acid, ergosterol or stigmasterol, but is preferably cholesterol.

If R_4 is a steroid radical, R_1 and R_2 are preferably hydroxyl and R_3 is hydrogen.

$Z^{\oplus}$ is as defined for formula IB and is preferably sodium or potassium.

In an anionic surfactant of the formula IC, m is preferably 1, R_1 is alkyl, e.g. n-dodecyl, (lauryl), n-tridecyl, n-tetradecyl (myristyl), n-pentadecyl, n-hexadecyl (cetyl), n-heptadecyl or n-octadecyl (stearyl), alkoxy, e.g. n-dodecyloxy (lauryloxy),

n-tetradecyloxy (myristyloxy), n-hexadecyloxy (cetyloxy), or n-octadecyloxy (stearyloxy), acyloxy, e.g. lauroyloxy, myristoyloxy, palmitoyloxy or stearyloxy, R_2 is hydrogen or hydroxy, R_3 is hydrogen or lower alkyl, e.g. methyl, R_4 is lower alkyl, e.g. methyl or ethyl, lower alkyl substituted by acid and basic groups, e.g. carboxy and amino, e.g. ω-amino-ω-carboxy-lower alkyl, e.g. 2-amino-2-carboxyethyl or 3-amino-3-carboxy-n-propyl, hydroxy-lower alkyl, e.g. 2-hydroxyethyl or 2,3-hydroxypropyl, lower alkylenedioxy-lower alkyl, e.g. 2,3-ethylenedioxypropyl or 2,3-(2,2-propylene)dioxypropyl, halo-lower alkyl, e.g. 2-chloroethyl or 2-bromoethyl, a carbohydrate radical of 5 to 12 carbon atoms, e.g. inositol, or a steroid radical, e.g. a sterol such as cholesterol, and $Z^{\oplus}$ is sodium or potassium.

An anionic surfactant of the formula IC is preferably the sodium or potassium salt of lysophosphatidylserine, e.g. the sodium or potassium salt of beef brain lysophosphatidylserine or the sodium or potassium salt of a synthetic lysophosphatidylserine, e.g. sodium or potassium 1-myristoyllysophosphatidylserine or sodium or potassium 1-palmitoyllysophosphatidylserine, or the sodium or potassium salt of lysophosphatidyl glycerol.

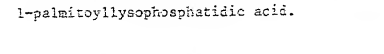
In an anionic surfactant of the formula ID, R_1, R_2, R_3 and $Z^{\oplus}$ are as defined for formula IC. The cation $Z^{\oplus}$ is preferably sodium or potassium. The hydrogen atom at the phosphate group may be replaced by a second cation $Z^{\oplus}$ or by the magnesium ion.

In an anionic surfactant of the formula ID, R_1 is preferably alkyl, e.g. n-dodecyl (lauryl), n-tridecyl, n-tetradecyl (myristyl), n-pentadecyl, n-hexadecyl (cetyl, n-heptadecyl or n-octadecyl (stearyl), or alkoxy, e.g. n-dodecyloxy (lauryloxy), n-tetradecyloxy (myristyloxy), n-hexadecyloxy (cetyloxy), or n-octadecyloxy (stearyloxy), or acyloxy, e.g. lauroyloxy, myristoyloxy, palmitoyloxy

or stearoyloxy, R_2 is hydrogen or hydroxy and R_3 is hydrogen or lower alkyl, e.g. methyl, and $2^{\oplus}$ is sodium or potassium.

An anionic surfactant of the formula ID is in particular the sodium or potassium salt of a natural phosphatidic acid, e.g. egg phosphatidic acid, the sodium or potassium salt of a natural lysophosphatidic acid, e.g. egg lysophosphatidic acid, the sodium or potassium salt of a synthetic lysophosphatidic acid. e.g. 1-lauroyl-lysophosphatidic acid, 1-myristoyllysophosphatidic acid or 1-palmitoyllysophosphatidic acid.

A lipid which is dispersed in the aqueous phase is e.g. a compound of the formula



(IC'),

wherein m, R_1, R_2, R_3 and R_4 are as defined for formula IC, and R_4 is also lower alkyl substituted by tri-lower alkylammonio, e.g. trimethylammonio, or by amino, e.g. 2-trimethylammonioethyl (cholinyl).

A suitable lipid is preferably a lipid of the formula IC', wherein m is 1, R_1 and R_2 are acyloxy, R_3 is hydrogen and R_4 is 2-trimethylammonioethyl or 2-aminoethyl. Such a lipid is e.g. a natural lecithin, e.g. egg lecithin or lecithin obtained from soybeans (R_4 is 2-trimethylammonioethyl), and a natural cephalin, e.g. egg cephalin or cephalin obtained from soybeans (R_4 is 2-aminoethyl).

Further preferred lipids are synthetic lecithins (R_4 = 2-trimethylammonioethyl) and synthetic cephalins (R_4 = 2-aminoethyl) of the formula IC', wherein R_1 and R_2 are identical acyloxy radicals such

as lauroyloxy, oleoyloxy, linoyloxy, linoleoyloxy or arachinoyloxy, e.g. dilauroyl lecithin or cephalin, dimyristoyl lecithin or cephalin, dipalmitoyl lecithin or cephalin, distearoyl lecithin or cephalin, diarachinoyl lecithin or cephalin, dioleoyl lecithin or cephalin, dilinoyl lecithin or cephalin, dilinoleoyl lecithin or cephalin, or diarachinoyl lecithin or cephalin, R_1 and R_2 are different acyloxy radicals, e.g. R_1 is palmitoyloxy and R_2 is oleoyloxy, e.g. 1-palmitoyl-2-oleoyl lecithin or cephalin, R_1 and R_2 are identical alkoxy radicals, e.g. tetradecyloxy or hexadecyloxy, e.g. ditetradecyl lecithin or cephalin, or dihexadecyl lecithin or cephalin, R_1 is alkenyl and R_2 is acyloxy, e.g. a plasmalogen (R_4 = trimethylammonioethyl), or R_1 is acyloxy, e.g. myristoyloxy or palmitoyloxy, and R_2 is hydroxy, e.g. a natural or synthetic lysolecithin or lysocephalin, e.g. 1-myristoyl lysolecithin or lysocephalin or 1-palmitoyl lysolecithin or lysocephalin, and R_3 is hydrogen.

A suitable lipid is also a lipid of the formula IC', wherein m is 1, R_1 is alkenyl, R_2 is acylamido, R_3 is hydrogen, and R_4 is a 2-trimethylammonioethyl radical (choline radical). Such a lipid is known as sphingomyelin.

A suitable lipid is furthermore a lysolecithin analogue, e.g. 1-lauroyl-1,3-propanediol-3-phosphorylcholine, a monoglyceride, e.g. monoolein or monomyristin, a cerebroside, a ganglioside or a glyceride which contains no free or etherified phosphoryl or phosphonyl groups in the 3-position. Such a glyceride is e.g. a diacylglyceride or 1-alkenyl-1-hydroxy-2-acylglyceride containing the indicated acyl and alkenyl groups, wherein the 3-hydroxy group is etherified by one of the indicated carbohydrate radicals, e.g. a galactosyl radical, e.g. a monogalactosyl glycerol.

Yet another suitable lipid is a neutral lipid which is contained in cell membranes and is soluble only in a polar organic solvent, e.g. in chloroform. Examples of neutral lipids are steroids such as oestradiol or sterol, e.g. cholesterol, β-sitosterol, desmosterol, 7-keto-cholesterol or β-cholestanol, fat-soluble vitamins such as vitamin A, e.g. vitamin A_1 or A_2, vitamin E, vitamin K such as vitamin K_1 or K_2, or vitamin D_2 or D_3.

The homogeneous mixture consists preferably of a surfactant of the formula IA, in particular N-benzyl-N,N-dimethyl-N-2-[2-(4-(1,1,3,3-tetramethylbutyl)phenoxy)ethoxy]ethylammonium chloride, N-benzyl N,N-dimethyl-N-2-[2-(3-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxy)-ethoxy]ethylammonium chloride (methylbenzethonium chloride), n-dodecyl-trimethylammonium chloride or bromide, trimethyl n-tetradecyl-ammonium chloride or bromide, n-hexadecyltrimethylammonium chloride or bromide (cetyltrimethylammonium chloride or bromide), trimethyl-n-octadecylammonium chloride or bromide, ethyl n-dodecyldimethyl-ammonium chloride or bromide, ethyldimethyl-n-tetradecylammonium chloride or bromide, ethyl n-hexadecyldimethylammonium chloride or bromide, ethyldimethyl n-octadecylammonium chloride or bromide, n-alkyl benzyldimethylammonium chloride or bromide (benzalkonium chloride or bromide), e.g. benzyl n-dodecyldimethylammonium chloride or bromide, benzyldimethyl n-tetradecylammonium chloride or bromide, benzyl n-hexadecyldimethylammonium chloride or bromide or benzyl-dimethyl-n-octadecylammonium chloride or bromide, N-(n-decyl)-pyridinium chloride or bromide, N-(n-dodecyl)pyridinium chloride or bromide, N-(n-tetradecyl)pyridinium chloride or bromide, N-(n-hexadecyl)pyridinium chloride or bromide (cetylpyridinium chloride or bromide), or N-(n-octadecyl)pyridinium chloride or bromide, or an anionic surfactant of the formula IB, in particular sodium or potassium n-dodecyl (lauryl) sulfate, sodium or potassium n-tetradecyl (myristyl) sodium or potassium n-hexadecyl (cetyl) sulfate or sodium or potassium n-octadecyl (stearyl) sulfate, sodium or potassium n-dodecyloxyethyl sulfate, sodium or potassium n-tetradecyloxyethyl

sulfate, sodium or potassium n-hexadexyloxyethyl sulfate or sodium or potassium n-octadecyloxyethyl sulfate, or an anionic surfactant of the formula IC, in particular sodium or potassium 2,2-dimethyl-3-palmitoyloxypropyl hydrogen phosphate, sodium or potassium 1-palmitoyllysophosphatidyl glycerol, sodium or potassium 1-palmitoyllysophosphatidylserine, and a lipid of the formula IC', wherein R_1 and R_2 are acyloxy, e.g. lauroyloxy, myristoyloxy, palmitoyloxy or stearoyloxy, R_3 is hydrogen and R_4 is 2-trimethylammonioethyl, e.g. a natural cephalin such as egg cephalin or cephalin or cephalin obtained from soybeans, or 2-aminoethyl, e.g. a natural lecithin such as egg lecithin or lecithin obtained from soybeans.

The surfactants and lipids containing a chiral carbon atom mentioned above and hereinafter may also be in the form of racemic mixtures or of optionally pure enantiomers.

In the homogeneous mixture, the approximate molar ratio of anionic surfactant to lipid is 0.1 to 2:1, preferably 0.8 to 1.2:1.

The homogeneous mixture, e.g. the prepared film or foam, is subsequently dispersed in an aqueous phase containing the substances to be encapsulated, e.g. agrochemicals such as pesticides, perfumes, hardeners, dyes, or pharmaceutical drugs such as peptides, e.g. muramyl peptides, in dissolved, colloidal or suspended form, and surfactants.

Dispersion is effected e.g. by shaking or stirring the aqueous phase which contains the previously prepared homogeneous mixture. The formation of unilamellar liposomes (SUL) and (LUL) takes place spontaneously (spontaneous vesiculation), i.e. without the additional supply of external energy and at a high rate. The concentration of surfactant, lipid and encapsulated compound can be increased until the critical micelle concentration (cmc) of the particular ionic

surfactant in the particular aqueous phase is attained.

Micelles are preferably formed above the critical micelle concentration. This occurrence is often detectable by the disappearance of opalescence, e.g. clarification of the aqueous phase. The cmc is a variable indicating the amount of an anionic surfactant which can be dispersed in a specific volume of water while avoiding micelle formation. The structure of the hydrophobic radical of the surfactant influences the cmc value: the longer the chain length, the lower the cmc value. Voluminous substituents in the hydrophobic radical, e.g. an aromatic radical, also lower the cmc. Functional groups, e.g. double bonds which weaken the hydrophobic character of the hydrophobic radical, increase the cmc. The cmc is further influenced by all dispersed and dissolved components present in the aqueous phase, e.g. by counterions, additional lipids, the character of the active ingredient to be encapsulated etc. The cmc value can only be determined experimentally for the particular system, namely indirectly by electrochemical methods, e.g. by conductivity measurements or potentiometric determination of the counterions using a suitable electrode, by measuring the transport number and the surface tension, by measuring colligative properties such as lowering of vapour pressure, lowering of the freezing point and osmotic pressure, measuring the density, the refractive index, the absorption of UV and IR light, solubilisation of soluble and insoluble dyes, light scattering, fluorescence polarisation and viscosity. These properties undergo a substantial change when the cmc is attained. For example, the surface tension decreases sharply as a function of the concentration of the ionic surfactant until the cmc is attained, but remains virtually constant above the cmc. Reference is made in this connection to the particulars given in H. Stache, Tensidtaschenbuch, Hanser 1981, especially on page 26, 3.1 "Methoden zur cmc-Bestimmung" and page 28, 3.2 "Abhängigkeit der cmc von verschiedenen Parametern." Specific cmc values, e.g. for dodecylpyridinium bromide, are given by J.B. Adderson and H. Taylor,

J. Colloid. Sci. 19, 495 (1964). If the cmc value is exceeded, it is possible to lower the concentration by diluting the aqueous phase with water. Reversibly unilamellar liposomes are then formed from the micelles.

Aqueous phases with a pH higher than about 8 are neutralised following dispersion, e.g. to physiological pH 7.2. Neutralisation is necessary to prevent decomposition of the active ingredient and/or the liposomes under basic conditions and to ensure the physiological tolerance of the applicable aqueous phase with the mixture of liposomes. Neutralisation is effected with a physiologically acceptable acid or a buffer solution with a pH of 7 to 8. Physiologically acceptable aicds are e.g. dilute mineral acids such as dilute hydrochloric acid, sulfuric acid or phosphoric acid, or organic acids such as lower alkanecarboxylic acids, e.g. acetic acid.

Aqueous phases containing cationic surfactants of the formula IA may show acid reaction. These phases are neutralised by adding dilute aqueous bases, e.g. dilute aqueous NaOH or KOH or a buffer solution with a pH of 7 to 8.

The process is conveniently carried out at room temperature or also at elevated temperature, e.g. up to about 60°C, and with stirring or shaking. If the limited stability of the active ingredient to be encapsulated requires it, the process is carried out with cooling and, if appropriate, in an inert gas atmosphere, e.g. in a nitrogen atmosphere. The liposomes so obtained are fairly stable in aqueous phase (up to several days). Aqueous phases containing unilamellar liposomes obtainable by the process of this invention can be made storage stable by the process described in European patent application 00 65 292.

The size of the unilamellar liposomes depends inter alia on the structure of the surfactants and of the lipid components, on the ratio of the components, on the concentration of these components in the aqueous phase, and on the amount and structure of the drug to be encapsulated. Accordingly, for example, aqueous phases containing a high concentration of small or large unilamellar liposomes can be prepared by varying the concentration of the surfactant components. In addition to SUL, large unilamellar liposomes (LUL, diameter up to 50,000 Å) are also formed. These encapsulate larger volumes per mole of lipid components employed and are suitable for encapsulating voluminous substances, e.g. viruses, bacteria or cell organellae.

The separation of SUL from LUL is accomplished by conventional separation methods such as gel filtration, e.g. with Sepharose 4B or Sephacryl as carrier, or by sedimentation of the LUL in an ultracentrifuge at 160,000 x g. For example, the LUL deposit after centrifugation for several hours, e.g. about 3 hours, in this gravitional field, whereas the SUL remain in dispersion and can be decanted. Complete separation of the LUL from the SUL is achieved after repreated centrifugation.

All liposomes having a diameter greater than 600 Å present in the aqueous phase, e.g. LUL or multilamellar liposomes, as well as non-encapsulated drugs and excess dispersed lipids, can also be separated by gel filtration, so making it possible to obtain an aqueous phase containing a fraction of SUL of relatively uniform size.

After the separation of large unilamellar liposomes (LUL) and multilamellar liposomes by one of the above methods, the formation of small unilamellar liposomes and their concentration in aqueous phase can be detected by different physical methods, e.g. by applying freeze fracture samples and thin layer samples to the electron

microscope or by X-ray diffraction, by dynamic light scattering, by mass analysis of the filtrate in an analytical ultracentrifuge, in particular by spectroscopy, e.g. in the nuclear resonance spectrum (NMR) (^{1}H, ^{13}C and ^{31}P). For example, sharp signals of narrow line within the nuclear resonance spectrum indicate the formation of unilamellar liposomes with a diameter smaller than about 1000 Å. Sharp signals at δ c. 0.89 ppm ($-CH_3$), δ c. 1.28 ppm ($-CH_2-$) and δ c 3.23 ppm ($-N(CH_3)_3$) are characteristic e.g. of unilamellar liposomes obtained by the process of this invention with phosphatidyl choline as constituent. In the nuclear resonance spectrum, such signals are typical of unilamellar liposomes and differ from mixed micelles, e.g. from phospholipids such as lecithin, and surfactants such as cetyltrimethylammonium bromide. A methyl signal at δ c. 0.89 ppm is characteristic of mixed micelles with these components, which signal is resolved to a triplet and has a substantially narrower line width than the methyl signal (singlet; also δ c. 0.89 ppm) which originates from unilamellar liposomes.

The liposomes obtainable by the process of this invention (SUL and LUL) are suitable carrier systems which, in aqueous phase, may be used for solubilising lipophilic substances, e.g. fat-soluble dyes, for stabilising substances which are sensitive to hydrolysis, e.g. prostaglandins, for encapsulating pesticides, e.g. for modifying the activity spectrum of dichlorphos, for encapsulating food additives, e.g. to modify the adsorption properties of vitamins or dyes, or for introducing encapsulated drugs, enzymes, antibodies, hormones, genes, viruses, vitamins or cell organellae into the cells of a cell culture.

Aqueous phases which contain the liposomes obtainable by the process of the invention with encapsulated durgs are delivery systems which are suitable, optionally after concentration or isolation of the liposomes, e.g. by ultracentrifugation, for therapeutic purposes for oral (p.o.), parenteral (i.v. or i.p.) or topical administration.

In oral administration, liposome-based delivery systems can protect a drug, e.g. insulin, which is unstable in the digestive tract, or improve its resorption. For oral administration, the liposome-containing aqueous phase can be mixed with pharmaceutically acceptable diluents or carriers or with conventional additives such as dyes or flavourings, and administered as a syrup or in the form of capsules.

For parenteral administration, liposome-based delivery systems can prolong the retention time e.g. of desferrioxamin (q.v. R.A. Guilemette et al., Lif Sci. 22 (4), 313-320, 1978) or gentamycin (q.v. W.M. Scheld et al., Clin. Res. 26, No. 1, 59 A, 1978), in an organism. The retention time of entrapped chelating agents, e.g. EDTA (ethylenediamintetraacetic acid), in organisms is prolonged in the same manner, so that heavy metals can be removed by chelation especially from the liver, spleen or kidneys (q.v. Rahmann et al., Science, Vol. 180, 300-302, 1973, and J. Lab. Clin. Med. 640-647, 1974). With liposome-based delivery systems it is possible to enrich drugs in the myocardium (q.v. Landesmann et al., Science, Vol. 198, 737-738, 1977). It is possible to enrich antiflammatory drugs, e.g. cortisol (q.v. Nature 271, No. 5643, 372-73, 1978) or protease inhibitors (q.v. Anal. Biochem. 89, No. 2, 400-07, 1978) in the synovial fluid, and cytostatic drugs in tumour tissue (q.v. the article entitled "Liposomes - Problems and promise as selective drug carriers" by Stanley B. Kaye in Cancer Treatment Reviews 8, 27-50, 1981, and the many references cited therein). Many chemotherapeutic drugs employed in cancer therapy are less toxic and better tolerated if they are encapsulated in liposomes, e.g. liposome-encapsulated Actinomycin D (q.v. Rahmann et al., Proceedings of the Society for Experimental Biology and Medicine 146, 1173-1176, 1974), Methotrexate (q.v. L.D. Lasermann et al., Proc. Natl. Acad. Sci. 77, No. 7, 4089-93, 1980), Vinblastin, Daunomycin or cytosin-arabinoside (q.v. Mühlensiepen et al., Cancer Res. 41, No. 5, 1602-07, 1981). Liposomes can be used for introducing e.g. enzymes, peptide hormones, genes or viruses into the cytoplasma of cells in

living organisms, e.g. for introducing asparaginase (q.v. the article entitled "The Introduction of enzymes into cells by means of liposomes" by M. Finkelstein and G. Weissmann in J. Lipid Research, Vol. 19, 1978, 289-303), of amyloglucosidase (q.v. G. Gregoriadis and B.E. Ryman, Eur. J. Biochem. 24 (1972), 485-491, or neuromidase (q.v. Gregoriadis et al., Biochem. J. (1974) 140, 232-330), for bonding specific detection molecules, e.g. monoclonal antibodies, for specific introduction into defined target cells (q.v. Lesermann et al., Nature 292 (5829), 226-228, 1981), for immunostimulation as adjuvant for inoculations, e.g. against leishmaniasis (q.v. New, R.R.C. et al., Nature 272 (5648) 55-56, 1978), or for the induced release of drugs by signals such as temperature increases, e.g. in inflamed tissue, or changes in pH values. For parenteral administration, the concentrated or isolated liposomes can be suspended in a suitable carrier liquid, for example in sterile distilled water or in physiological sodium chloride solution.

Preparation of the homogeneous layer of lipid components

The homogeneous layer of lipid components can be prepared in a manner which is known per se. For example, the surfactant of the formula IA, e.g. cetylpyridinium chloride, and the lipid, e.g. egg lecithin, optionally in admixture with a lipophilic active ingredient, e.g. a protein which is encapsulated during the formation of the liposome in the lipid layer, is dissolved in an organic solvent. A homogeneous layer of lipid components consisting of a film is obtained by removing the organic solvent, most conveniently in vacuo or by blowing off with an inert gas, e.g. nitrogen.

The choice of solvent depends on the solubility of the particular lipid components therein. Examples of suitable solvents are: halogenated, aliphatic, cycloaliphatic, aromatic or aromatic-aliphatic hydrocarbons, e.g. benzene, toluene, methylene chloride or chloroform; alcohols, e.g. methanol or ethanol; lower alkane-carboxylates, e.g. ethyl acetate; ethers, e.g. diethyl ether, dioxan

or tetrahydrofuran; or mixtures of these solvents.

A homogeneous mixture can be prepared by the manner described in German Auslegeschrift 28 18 655 by lyophilisation from organic solution. The homogeneous layer is obtained as a foam.

The ionic surfactants mentioned in the description, e.g. the cationic surfactants of the formula IA and the anionic surfactants of the formula IB are known. The preparation of these surfactants is described in the standard work "Cationic Surfactants" by Eric Jungermann, Dekker, New York 1970. The annually published handbook "McCutcheon's Emulsifiers & Detergents", Manufacturing Confectioner Publishing Co., provides a survey of all commercially available anionic and cationic surfactants together with the trade names under which these surfactants are marketed by the manufacturers. The surfactants of the formulae IB and IC are known or, if novel, can be prepared in a manner known per se in accordance with the particulars given in Chapter 3 of the standard work by G.G. Knight, Liposomes, Elsevier 1981. The lipids referred to hereinbefore are known and most are commercially available.

The following Examples illustrate the invention, without implying any restriction to what is disclosed therein. Chemical displacements (δ) in the NMR spectrum are indicated in ppm.

Example 1: 10 mg of egg lecithin and 0.05 g of cetyltrimethylammonium bromide are dissolved in 2 ml of a 2:1 mixture of chloroform/methanol and this solution is concentrated in vacuo by rotary evaporation. Unilamellar liposomes are formed by dispersing the film-like residue at room temperature in 1 ml of water by shaking for 5-10 minutes. A slightly opalescent aqueous phase is obtained.

The formation of small unilamellar liposomes can be detected in the NMR spectrum by the signals δ = 1.28 (methylene), δ = 0186 (methyl) and δ = 3.25 ($-N(CH_3)_3$).

The unilamellar liposomes so obtained can be made visible in an electron microscope. The liposome dispersion is first subjected to conventional freeze-fracture. There are obtained mainly two "populations" of liposomes, which differ in their average size:

1. small unilamellar liposomes (SUL) with a diameter of about 200-600 Å and

2. large unilamellar liposomes (LUL) with a diameter of about 1000-10,000 Å.

Example 2: Following the procedure of Example I, 10 mg of egg lecithin and an increasing amount of cetyltrimethylammonium bromide (CTAB, see table 1) are dissolved in 2 ml of a 2:1 mixture of chloroform/methanol. The solution is concentrated and the residue is dispersed in water to give an opalescent aqueous phase which consists of small (SUL) and large (LUL) unilamellar liposomes.

Table 1:

Experiment	Concentration CTAB [g/l]	Yield of SUL [%]
1	0.1	10
2	0.2	10
3	0.5	10
4	1.0	10
5	2.0	12
6	5.0	14
7	7.0	20
8	10.0	40
9	15.0	70

Example 3: In each experiment, 10 mg of egg lecithin and an increasing amount of cetylpyridinium chloride (CPC, see Table 2) or benzalkonium chloride (BAS, see Table 3) are dissolved in 2 ml of a 3:1 mixture of chloroform/methanol. The solution is concentrated in vacuo and the residue is dispersed in 1 ml of water by shaking for 5-10 minutes to give an opalescent aqueous phase which consists of small (SUL) and large (LUL) unilamellar liposomes.

Table 2:

Experiment	Concentration CPC [g/l]	Yield of SUL [%]
1	1.0	10
2	1.5	15
3	2.0	20
4	2.5	20
5	3.0	25
6	3.5	30

Table 3:

Experiment	Concentration BAC [g/l]	Yield of SUL [%]
1	0.5	2
2	1.0	5
3	2.0	5
4	3.0	10
5	5.0	15
6	10.0	60

Example 4: In each experiment, 10 mg of egg lecithin and an increasing of Texapon N 25 ® (sodium lauryl ether sulfate, see Table 4), octadecylphospho-D-mannitol (OPM, see Table 5) or sodium

dodecyl sulfate (SDS, see Table 6) are dissolved in 2 ml of a 2:1 mixture of chloroform/methanol. The solution is concentrated in vacuo and the residue is dispersed in 1 ml of water by shaking for 5-10 minutes to give an opalescent aqueous phase which consists of small (SUL) and large (LUL) unilamellar liposomes.

Table 4:

Experiment	Concentration Texapon N 25 [g/l]	Yield of SUL (%)
1	1.0	2
2	2.0	5
3	3.0	5
4	4.0	10

Table 5:

Experiment	Concentration OPM [g/l]	Yield of SUL [%]
1	1.0	10
2	2.0	15
3	3.0	20

Table 6:

Experiment	Concentration OPM [g/l]	Yield of SUL [%]
1	1.0	5
2	2.0	8
3	3.0	10
4	4.0	12
5	5.0	15
6	6.0	15
7	7.0	20
8	8.0	30
9	9.0	35

Example 5: A total amount of 10 mg containing the amount indicated in Table 3 of sodium 2,2-dimethyl-3-palmitoyloxypropyl hydrogen phosphate (Table 7), sodium 1-palmitoyllysophatidyl glycerol (Table 8) and sodium 1-palmitoyllysophosphatidylserine (Table 9) and the corresponding amount of egg lecithin (lipid) are dissolved in 1 ml of a 2:1 mixture of chloroform/methanol and the solution is concentrated by rotary evaporation. The film-like residue is then dispersed in 1 ml of distilled water and the dispersion is neutralised with 0.1N sodium hydroxide solution. An opalescent aqueous phase is obtained.

Table 7:

Experiment	Concentration Surfactant [g/l]	Yield of SUL [%]
1	0.5	7
2	1.0	13
3	1.5	19
4	2.0	23
5	2.5	26
6	3.0	30
7	4.0	37
8	5.0	60
9	6.0	83
10	7.0	90
11	8.0	95
12	9.0	100
13	9.5	100

Table 8:

Experiment	Concentration Surfactant [g/l]	Yield of SUL [%]
1	1.0	6
2	1.5	10
3	2.0	15
4	2.5	17
5	3.0	20
6	3.5	25
7	4.0	27
8	4.5	30
9	5.0	33
10	6.0	40

Table 9:

Experiment	Concentration Surfactant [g/l]	Yield of SUL [%]
1	1	5
2	2	8
3	3	13
4	4	18
5	5	20
6	6	25

Example 6: 3 mg of one of the surfactants listed in Table 10 and 7 mg of egg lecithin (lipid) are dissolved in 1 ml of a 2:1 mixture of chloroform/methanol and the solution is concentrated. The film-like residue is dispersed in 1 ml of water and the dispersion is neutralised with 0.1N NaOH. An opalescent aqueous phase is obtained.

Table 10:

Surfactant	Yield [% SUL]
2-hydroxyethyl-3-palmitoyloxypropyl phosphate	20
2,2-dimethyl-3-palmitoyloxypropyl hydrogenphosphate	50
3-cetyloxypropyl-2-hydroxyethyl phosphate	29
2-bromoethylcetylphosphate	30
n-eicosyl-2,3-(2,2-propylene)dioxypropyl phosphate	18
3-stearyloxypropylhydrogen phosphate	8
2,3-dihydroxypropylmyristyl phosphate	34
3-cetyloxypropylhydrogen phosphate	19
2,3-dihydroxypropyl-n-eicosyl phosphate	8
cetyl 2,3-dihydroxypropyl phosphate	25
methyl 3-stearoyloxypropyl phosphate	45

Example 7: 20 mg (0.026 mmole) of soybean lecithin, 1 mg (0.76 umole) of N-acetylmuramyl-L-alanyl-2-(1',2'-dipalmitoyl-sn-glycero-3'-phosphoryl)ethylamide and 5 mg of n-hexadecylpyridinium chloride are dissolved in 2 ml of a 2:1 mixture of chloroform/methanol and the solution is concentrated by rotary evaporation. The film-like residue is shaken for 5 minutes in 3 ml of distilled water to give an opalescent aqueous phase. The aqueous dispersion is then buffered with 0.2 ml of a 10-fold concentrate of a phosphate-buffered isotonic solution of sodium chloride (PBS for injection purposes) to pH 7.4.

Example 8: 30 mg (0.04 mmole) of soybean lecithin, 2 mg (0.004 mmole) of flumethason 21-pivalate and 8 mg (0.002 mmole) of n-hexadecyl-pyridinium chloride are dissolved in 2 ml of a 2:1 mixture of chloroform/methanol and the solution is concentrated by rotary evaporation. The film-like residue is shaken for 5 minutes in 3 ml of distilled water to give an opalescent aqueous phase. The aqueous dispersion is then buffered to pH 7.4 as described in Example 7.

Example 9: 30 mg (0.040 mmole) of soybean lecithin and 15 mg (0.042 mmole) of Lanette E® (sodium stearyl or palmityl sulfate) are dissolved in 8 ml of a 4:1 mixture of tert.-butanol/methanol at 70°C and the solution is concentrated in vacuo. The film-like residue is shaken for 5 minutes in 3 ml of distilled water to give an opalescent aqueous phase which is buffered to pH 7.4 as described in Example 7.

Example 10: 20 mg (0.026 mmole) of soybean lecithin, 1 mg (0.76 mmole) of N-acetylmuramyl-L-alanyl-2-(1',2'-dipalmitoyl-sn-glycero-3'-phosphoryl)ethylamide and 10 mg of (0.028 mmole) of Lanette E® are dissolved in 6 ml of 4:1 mixture of tert-butanol/methanol and the solution is concentrated by rotary evaporation. The film-like residue is shaken for 5 minutes in 2 ml of distilled water to give an opalescent aqueous phase. The aqueous dispersion is filled into a stirred ultrafiltration cell (Amicon®), which, instead of the ultrafilter, is provided with an even pore filter of polycarbonate (Nucleopore®) which has a pore diameter of 0.1 um, and has been washed free of particles. The dispersion is filtered under slight overpressure and with constant addition of Dulbecco's sterile buffer solution (pH 7.4 without Ca and Mg) so that the volume in the cell does not decrease to less than 30 ml. After the passage of 0.3 litre of filtrate, all the SUL are separated and the supernatant dispersion of LUL can be filled into ampoules and used for treatment assays.

Example 11: 30 mg (0.04 mmole) of soybean lecithin. 4 mg (0.081 mmole) of flumethason 21-pivalate and 10 mg (0.028 mmole) of Lanette E® are dissolved in 6 ml of a 4:1 mixture of tert-butanol/methanol at about 70°C and the solution is concentrated by rotary evaporation. The film-like residue is shaken in 3 ml of distilled water to give an opalescent aqueous phase.

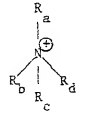
The dispersion is filled into a stirred filter cell (total volume: 100 ml) as described in Example 10 and then filtered, while adding sterile water which has been filtered until free of particles, until 500 ml of filtrate have collected. This filtrate is fed continuously into a stirred filter cell equipped with an ultrafilter, e.g. Amicon U 10 ®, and then concentrated to a volume of 30 ml. The concentrated dispersion contains small unilamellar liposomes and, after addition of Dulbecco's phosphate buffer (pH 7.4, without Ca and Mg), is filled into ampoules and used for treatment assays.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A process for the preparation of unilamellar liposomes in aqueous phase, which comprises dispersing a homogeneous mixture of an anionic surfactant and a lipid, in aqueous phase, at a concentration lower than the critical micelle concentration (cmc) of the surfactant in the particular phase and, if necessary, neutralising the aqueous phase so obtained and, if desired, enriching and/or separating the resultant unilamellar liposomes.

2. A process according to claim 1, which comprises dispersing a homogeneous mixture of an anionic or cationic surfactant.

3. A process according to claim 2, which comprises dispersing a homogeneous mixture of a cationic surfactant of the formula



(IA),

wherein R_a is an unsubstituted or substituted hydrocarbon radical, R_b is lower alkyl, phenyl-lower alkyl or hydroxy, R_c and R_d are lower alkyl, or R_b and R_c, together with the nitrogen atom to which they are attached, form an aliphatic heterocyclic ring system which may be substituted at a carbon atom, and R_d is lower alkyl, or R_b, R_c and R_d, together with the nitrogen atom to which they are attached, form an aromatic heterocyclic ring system, and $Y^{\ominus}$ is an anion, and a lipid.

4. A process according to claim 3, which comprises dispersing a homogeneous mixture of N-benzyl-N,N-dimethyl-N-2-[2-(4-(1,1,3,3-tetramethylbutyl)phenoxy)ethoxy]ethyl-

ammonium chloride, N-benzyl N,N-dimethyl-N-2-[2-(3-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxy)ethoxy]ethylammonium chloride (methylbenzethonium chloride), n-dodecyltrimethylammonium chloride or bromide, trimethyl n-tetradecylammonium chloride or bromide, n-hexadecyltrimethylammonium chloride or bromide (cetyltrimethylammonium chloride or bromide), trimethyl n-octadecylammonium chloride or bromide, ethyl n-dodecyldimethylammonium chloride or bromide, ethyldimethyl n-tetradecylammonium chloride or bromide, ethyl n-hexadexyldimethylammonium chloride or bromide, ethyldimsthyl n-octadecylammonium chloride or bromide, n-alkyl benzyldimethylammonium chloride or bromide (benzalkonium chloride or bromide), e.g. benzyl n-dodecyldimethylammonium chloride or bromide, benzyl n-hexadecyldimethylammonium chloride or bromide or benzyldimethyl n-octadecylammonium chloride or bromide, N-(n-decyl)pyridinium chloride or bromide, N-(n-dodecyl)pyridinium chloride or bromide, N-(n-tetradecyl)pyridinium chloride or bromide, N-(n-hexadecyl)pyridinium chloride or bromide (cetylpyridinium chloride or bromide), or N-(n-octadecyl)pyridinium chloride or bromide, or a mixture of these surfactants, and a lipid.

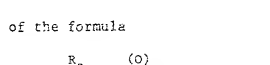
5. A process according to claim 2, which comprises dispersing a homogeneous mixture of an anionic surfactant

a) of the formula

$$[R_a-(O-A)_m-B]^{\ominus}Z^{\oplus} \qquad (IB)$$

wherein R_a is an unsubstituted or substituted hydrogen radical, A is lower alkylene, m is 0 (direct bond) or 1, B is the sulfonate or sulfate group and $Z^{\oplus}$ is a monovalent cation, or

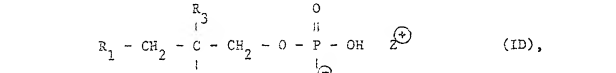
b) a compound of the formula



(IC),

wherein m is 0 or 1, one of R_1 and R_2 is hydrogen, hydroxy or lower C_1-C_4alkyl, and the other is alkyl, alkenyl, alkoxy, alkenyloxy or acyloxy, each of 10 to 20 carbon atoms, R_3 is hydrogen or lower C_1-C_4alkyl, and R_4 is unsubstituted or substituted lower C_1-C_7alkyl, a carbohydrate radical of 5 to 12 carbon atoms or, if both R_1 and R_2 are hydrogen or hydroxy, is a steroid radical, and $Z^{\oplus}$ is a monovalent cation, or

c) a compound of the formula



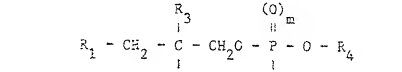
(ID),

wherein R_1, R_2, R_3 and $Z^{\oplus}$ are as defined for formula (IC), and a lipid.

6. A process according to claim 5, which comprises dispersing a homogeneous mixture containing an alkali metal alkyl sulfate (m = 0), e.g. sodium or potassium n-dodecyl (lauryl) sulfate, sodium or potassium n-tetradecyl (myristyl) sulfate, sodium or potassium n-hexadecyl (cetyl) sulfate or sodium or potassium n-octadecyl (stearyl) sulfate, an alkali metal alkyl ether sulfate (m = 1), e.g. sodium or potassium n-dodecyloxyethyl sulfate, sodium or potassium n-tetradecyloxyethyl sulfate, sodium or potassium n-hexadecyloxyethyl sulfate or sodium or potassium n-octadecyloxyethyl sulfate, or an alkali metal alkane sulfonate, e.g. sodium or potassium n-dodecane sulfonate, sodium or potassium n-tetradecane sulfonate, sodium or potassium n-hexadecane sulfonate or sodium or potassium n-octadecane sulfonate, the sodium or potassium salt of lyso-phosphatidylserine, e.g. the sodium or potassium salt of beef brain

lysophosphatidylserine or the sodium or potassium salt of a synthetic lysophosphatidylserine, e.g. sodium or potassium 1-myristoyllysophosphatidylserine or sodium or potassium 1-palmitoyllysophosphatidylserine, or the sodium or potassium salt of lysophosphatidylglycerol, the sodium or potassium salt of natural phosphatidic acid, e.g. egg phosphatidic acid, the sodium or potassium salt of a natural lysophosphatidic acid, e.g. egg lysophosphatidic acid, the sodium or potassium salt of a synthetic lysophosphatidic acid, e.g. 1-lauroyllysophosphatidic acid, 1-myristoyllysophosphatidic acid or 1-palmitoyllysophosphatidic acid, or a mixture of these surfactants and a lipid.

7. A process according to any one of claims 1 to 6, wherein the lipid is a compound of the formula



(IC'),

wherein m, R_1, R_2, R_3 and R_4 are as defined for formula IC and R_4 is also lower alkyl substituted by tri-lower alkylammonio or amino.

8. A process according to claim 7, wherein the lipid is preferably a natural lecithin, for example egg lecithin or lecithin obtained from soybeans (R_4 = 2-trimethylammonioethyl), a natural cephalin, for example egg cephalin or cephalin obtained from soybeans (R_4 = 2-aminoethyl), a synthetic lecithin (R_4 = 2-trimethylammonioethyl) or a synthetic cephalin (R_4 = 2-aminoethyl) of the formula IC', wherein R_1 and R_2 are identical acyloxy radicals such as lauroyloxy, oleoyloxy, linoyloxy, linoleoyloxy or arachinoyloxy, e.g. dilauroyl lecithin or cephalin, dimyristoyl lecithin or cephalin, dipalmitoyl lecithin or cephalin, distearoyl lecithin or cephalin, diarachinoyl lecithin or cephalin, dioleoyl lecithin or cephalin, dilinoyl lecithin or cephalin, dilinoleoyl lecithin or

cephalin, or diarachinoyl lecithin or cephalin, R_1 and R_2 are different acyloxy radicals, e.g. R_1 is palmitoyloxy and R_2 is oleoyloxy, e.g. 1-palmitoyl-2-oleoyl lecithin or cephalin, R_1 and R_2 are identical alkoxy radicals, e.g. tetradecyloxy or hexadecyloxy, e.g. ditetradecyl lecithin or cephalin, or dihexadecyl lecithin or cephalin, R_1 is alkenyl and R_2 is acyloxy, e.g. a plasmalogen (R_4 = trimethylammonioethyl), or R_1 is acyloxy, e.g. myristoyloxy or palmitoyloxy, and R_2 is hydroxy, e.g. a natural or synthetic lysolecithin or lysocephalin, e.g. 1-myristoyl lysolecithin or lysocephalin or 1-palmitoyl lysolecithin or lysocephalin, and R_3 is hydrogen.

9. A process according to any one of claims 1 to 8, which comprises dispersing a homogeneous mixture of a surfactant of the formula IA, in particular N-benzyl N,N-dimethyl-N-2-[2-(4-(1,1,3,3-tetramethylbutyl)phenoxy)ethoxy]ethylammonium chloride, N-benzyl N,N-dimethyl-N-2-[2-(3-methyl-4-(1,1,3,3-tetramethylbutyl)phenoxy)-ethoxy]ethylammonium chloride (methylbenzethonium chloride), n-dodecyl-trimethylammonium chloride or bromide, trimethyl n-tetradecyl-ammonium chloride or bromide, n-hexadecyltrimethylammonium chloride or bromide (cetyltrimethylammonium chloride or bromide), trimethyl-n-octadecylammonium chloride or bromide, ethyl n-dodecyldimethyl-ammonium chloride or bromide, ethyldimethyl-n-tetradecylammonium chloride or bromide, ethyl n-hexadecyldimethylammonium chloride or bromide, ethyldimethyl n-octadecylammonium chloride or bromide, n-alkyl benzyldimethylammonium chloride or bromide (benzalkonium chloride or bromide), e.g. benzyl n-dodecyldimethylammonium chloride or bromide, benzyldimethyl n-tetradecylammonium chloride or bromide, benzyl n-hexadecyldimethylammonium chloride or bromide or benzyl-dimethyl-n-octadecylammonium chloride or bromide, N-(n-decyl)-pyridinium chloride or bromide, N-(n-dodecyl)pyridinium chloride or bromide, N-(n-tetradecyl)pyridinium chloride or bromide, N-(n-hexa-decyl)pyridinium chloride or bromide (cetylpyridinium chloride or

bromide), or N-(n-octadecyl)pyridinium chloride or bromide, or an anionic surfactant of the formula IB, in particular sodium or potassium n-dodecyl (lauryl) sulfate, sodium or potassium n-tetradecyl (myristyl) sodium or potassium n-hexadecyl (cetyl) sulfate or sodium or potassium n-octadecyl (stearyl) sulfate, sodium or potassium n-dodecyloxyethyl sulfate, sodium or potassium n-tetradecyloxyethyl sulfate, sodium or potassium n-hexadexyloxyethyl sulfate or sodium or potassium n-octadecyloxyethyl sulfate, or an anionic surfactant of the formula IC, in particular sodium or potassium 2,2-dimethyl-3-palmitoyloxypropyl hydrogen phosphate, sodium or potassium 1-palmitoyllysophosphatidyl glycerol, sodium or potassium 1-palmitoyllysophosphatidylserine, and a lipid of the formula IC', wherein R_1 and R_2 are acyloxy, e.g. lauroyloxy, myristoyloxy, palmitoyloxy or stearoyloxy, R_3 is hydrogen and R_4 is 2-trimethylammonioethyl, e.g. a natural cephalin such as egg cephalin or cephalin or cephalin obtained from soybeans, or 2-aminoethyl, e.g. a natural lecithin such as egg lecithin or lecithin obtained from soybeans.

10. A process according to any one of claims 1 to 9, which comprises dispersing a homogeneous mixture of a surfactant and a lipid according to claim 9, and a pharmaceutical drug.

11. A process according to any one of claims 1 to 10, which comprises dispersing a homogeneous mixture of an anionic surfactant of the formula IB, egg lecithin and a muramyl peptide.

12. A process according to any one of claims 1 to 10, which comprises dispersing a homogeneous mixture of a cationic surfactant of the formula IA, soybean lecithin and a muramyl peptide.

13. A process according to claim 12, which comprises dispersing a homogeneous mixture of n-hexadecylpyridinium chloride, soybean lecithin and N-acetylmuramyl-L-alanyl-2-(1',2'-dipalmitoyl-sn-glycero-3'-phosphoryl)ethylamide.

14. A delivery system based on liposomes for encapsulated N-acetyl-muramyl-L-alanyl-D-isoglutamyl-L-alanyl-2-(1',2'-dipalmitoyl-sn-glycero-3'-phosphoro)ethylamide, prepared by the process as claimed in claim 1.

15. A pharmaceutical composition containing a delivery system based on liposomes for encapsulated drugs as claimed in claim 14, in combination with pharmaceutically acceptable adjuvants.

16. A delivery system according to claim 14 for use in the treatment of humans or animals.

17. A pharmaceutical composition according to claim 14 for use in the treatment of humans or animals.

18. A method of treating diseases in humans or animals, which comprises the use of a delivery system as claimed in claim 14.

DATED this 27th day of July 1983.

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(54) LIPOSOME INCLUDING ACTIVE SUBSTANCE

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LIPOSOME INCLUDING ACTIVE SUBSTANCE

ABSTRACT OF THE DISCLOSURE

Liposome prepared by using a phospholipid and oily substance as a base material for the liposome including an active substance in the vesicle of the liposome is disclosed, the liposome having a wall membrane of high strength and being excellent in slowly releasing the included active substance therewithin to outside therefrom.

BACKGROUND OF THE INVENTION:

The present invention relates to liposomes including an active substance, particularly a physiologically active substance.

Hitherto, in cases where a physiologically active substance such as a medicine is directly administered into a living body, there have been many occasions causing (1) immunological problems of proliferation of the antibody to the medicine within the body, (2) problems of augumented side effects of the medicine resulting from the uptake of the medicine by the tissues other than the target of the medicine, or inversely, (3) problems caused by the inability of the medicine of passing through the target tissue, and further (4) problems caused by the inability of the medicine of maintaining its activity owing to its degradation, inactivation, etc. by enzymes within the living body.

However, it is considered that the above-mentioned problems will be resolved by administering the physiologically active substance such as a medicine loaded on a carrier which is able to transfer the active substance directly into the target tissue within the living body while protecting the active substance.

From the above-mentioned viewpoint, Japanese Patent Application Laying Open No. 118826/74 (DE-OS-22 49 552) has recently proposed a liposome comprising a vesicle having a closed lamellar structure (micelle) composed of at least one

bimolecular layer formed by a compound, represented by the general formula, X-Y, wherein X represents a polar and hydrophilic group such as phosphate, carboxyl, amino, hydroxyl or choline and Y represents a non-polar and hydrophobic group such as alkyl, alkenyl or alkynyl, for example, a purified phospholipid such as lecithin, phosphatidylethanolamine, phosphatidylserine, etc. as a material for forming the membrane of the above-mentioned layer and containing a physiologically active substance dissolved in an aqueous solution within the vesicle of the liposome. Since the wall membrane which forms the liposome protects the active substance in the aqueous solution within the vesicle even under severe conditions, for instance, those in gastrointestinal tracts, the activity of the active substance is not spoiled even when the liposome is orally administered. In addition, since the permeability of the liposome to a tissue of living body changes depending on its particle size (diameter), it is possible to raise the permeability of the liposome to the tissue by adjusting the diameter of the liposome. Accordingly, the above-mentioned liposome has been given attention as a possible means of selectively supplying a physiologically active substance contained in the liposome into a specified tissue of a living body.

However, the above-mentioned wall membrane which forms the above-proposed liposome and is consisted of pure phospholipid has defects of lack of pliability and of insufficient mechanical strength. In addition, owing to the excessively large effluent

velocity of the physiologically active substance contained in the vesicle to the outside, the liposome is not necessarily satisfactory concerning its property of releasing the above-mentioned physiologically active substance slowly within the living body, that is, so-called the slow-releasing property. Particularly, the above-mentioned liposome has a defect that the effluent velocity of the above-mentioned active substance is highly raised at a temperature higher than the transition temperature of the wall membrane which forms the above-mentioned liposome.

In order to improve the strength of the wall membrane of the above-mentioned liposome, a method is well-known in which a steroid lipid such as cholesterol is admixed with the above-mentioned phospholipid as the material for forming the membraneous layer of the liposome. Although the strength of the wall membrane forming the liposome is somewhat improved by the proposed method, the so-called slow-releasing property is not so much improved.

In consideration of the above-mentioned situation, the inventor of the present invention, after studying the methods for supplying the liposome having a strong wall membrane and having a favorable slow releasing property of the physiologically active substance into the living body, has found that the wall membrane of the liposome which was formed by a phospholipid containing molecules of oily substance such as "crude lecithin" has a higher pliability and is more excellent in the slow-releasing property of the physiologically active substance into the living body as compared to the wall membrane of the

conventional and publicly known liposome formed by using purified phospholipid. Accordingly, it is surprising to see that the liposome formed by using a phospholipid containing molecules of oily substance has the above-mentioned specific properties.

Accordingly, the purpose of the present invention is to provide a liposome containing physiologically active substance, of which the wall membrane is strong enough and which has a favorable slow-releasing property of the above-mentioned active substance within the living body.

The other purposes of the present invention will be made clear from the following description:

BRIEF EXPLANATION OF THE DRAWINGS:

FIGURE 1 shows the schematic illustration of the liposome of the present invention and FIGURE 2 shows a relationship between the amount of molecules of oily substance contained in the membraneous material forming the liposome according to the present invention containing glucose in the aqueous solution within the vesicle of the liposome and the percentage of the amount of glucose entrapped within the vesicle to the total amount of glucose used for forming the above-mentioned liposome loading glucose (briefly expressed hereinafter as the rate of entrapment). FIGURE 3 shows a comparison of permeability of the above-mentioned glucose from the liposome of the present invention and from the liposome shown in Comparative Example. FIGURE 4 shows a comparison of the persistency of hypoglycemic

action of insulin in a living body, which is supplied by the liposome of the present invention containing insulin therewithin to that by the liposome of Comparative Example also containing insulin therewithin. FIGURES 5 to 8 show the slow-releasing property to an active substances included within the respective liposomes of the present invention.

Among them, FIGURE 5 shows the change of the amount of radio-isotope in blood with the passage of time after the subcutaneous injection of the liposome prepared by using the above-mentioned substance, however, radio-labelled, as the active substance, and FIGURE 6 shows the change of the amount of radio-isotope in urine with the passage of time after the same treatment as above. FIGURE 7 shows the change of the residual amount of radio-isotope, at the site where the free radio-labelled active substance was directly injected subcutaneously as it is, with the passage of time, and FIGURE 8 shows the change of the residual amount of radio-isotope, at the site where the liposome including the radio-labelled active substance within its vesicle was injected subcutaneously, with the passage of time.

DETAILED DESCRIPTION OF THE INVENTION:

The characteristic feature of the present invention is, on the formation of a liposome including an active substance within its vesicle surrounded by a micellar membraneous layer, the use a material comprising micellar membraneous layer of phospholipid, in which molecules of a fatty substance are present

as the wall membrane of the above-mentioned liposome.

Another characteristic feature of the liposome, including an active substance, formed by using the above-mentioned material according to the present invention is its W/O/W-type complex emulsified state as shown in FIGURE 1 and its excellent property of slowly releasing the above-mentioned active substance included within its vesicle to the outside when taken into a living body. In FIGURE 1, X represents a hydrophilic group, Y a hydrophobic group, P a vesicle containing an aqueous solution. A molecules of fatty substance and Q an aqueous solution outside of liposome, respectively.

The material for forming the liposome of the present invention, as has been described above, comprises phospholipid with which molecules of a fatty substance are mixed or to which they are bonded. The phospholipid used herein is not particularly limited provided it has been used as the material for a membraneous layer of the conventional liposome, for instance, a single compound of lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserin, phosphatidylinositol, sphingomyelin, cardiolipin, etc. or its mixture, and according to the necessity, a sterol compound such as cholesterol may be contained.

The fatty substance present in the above-mentioned phospholipid is triglyceride, wax or mineral oil or the mixture thereof, for instance, those selected from vegetable oil such as soy-bean oil, cotton seed oil and sesame oil, and also from

mineral oils of coal-origin or petroleum-origin.

Particularly preferable is the single use of crude lecithin or the use of a mixture of crude lecithin and other oily substance mentioned above.

The term "crude lecithin" herein used means a fraction which eluates with chloroform or a mixture of chloroform and methanol in a ratio of 100 : 1 to 3 : 2 when a component rich in phospholipid derived from substance such as yolk and soybean oil is fractioned by column chromatography using alumina as a column material, the fraction consisting of 97 to 80% by weight of pure lecithin and 3 to 20% by weight of oily substance, for instance, triglyceride and carotenoid.

In cases where the liposome of the present invention is prepared by using the above-mentioned crude lecithin as a material for membraneous layer, the content of the oily substance in crude lecithin is adjusted so that the content of the fatty substance in the thus formed wall membrane becomes to 3 to 20% by weight, preferably 5 to 15% by weight. In addition, care should be taken to keep the content of the fatty substance in the above-mentioned wall membrane not over 20% by weight or the formation of the wall membrane is impaired to have reduced yield of the liposome. On the other hand, in the case where the content of the fatty substance is less than 3% by weight, the above-mentioned object of the present invention cannot be attained.

By the way, on the formation of the liposome according to the present invention, sterols such as cholesterol and

ergosterol and a substance capable of changing the electrically loaded state of the surface of the liposome, for instance, phosphatidic acid, dicetyl phosphate or ganglioside of bovine brain for giving negative charge, or stearylamine for giving positive charge may be admixed with the material for membraneous layer as the third component. The amount of addition of the third component may be determined adequately according to the property of the phospholipid suitably, and usually is 0 to 10% by weight of the material for membraneous layer.

In the case of forming the liposome of the present invention while using a mixture of the above-mentioned phospholipid and the oily substance as a material for membraneous layer, the conventional method for preparing a liposome is possibly applied. For instance, a method in which a thin film is formed from the above-mentioned material and after bringing the thus formed membrane into contact with a continuous phase containing an active substance to form a dispersion by agitation, a supersonic vibration is applied on the dispersed system, a method in which after mixing a solution of the above-mentioned material for membraneous layer in a solvent insoluble in water with an aqueous solution containing the above-mentioned (water-soluble) active substance, the mixture is subjected to a supersonic treatment for forming a precursor of liposome and then the solution containing the precursor is subjected to ultracentrifugal treatment in the co-presence of an aqueous medium, or a method in which after coating the surface of glass-beads and the like

with the above-mentioned material for membraneous layer, the coated beads are mixed with an aqueous solution containing the above-mentioned active substance to disperse the coated beads into the solution is applicable.

The amount of the above-mentioned material for membraneous layer on forming the above-mentioned liposome is 1 to 500 mg/ml of the liquid in which the liposome is suspended.

The membraneous layer of the liposome obtained by the above-mentioned method is formed as a result of mutual action between the hydrophobic group possessed by the phospholipid in the material for membraneous layer which forms the liposome and the molecules of oily substance which are present in the material for membrane layer. Accordingly, the morphological state of the liposome of the present invention is to be said quite different substantially from the state of the conventional liposome comprising the micelle of purified phospholipid.

In addition, the oily substance as a constituent of the wall membrane of the liposome according to the present invention, on the formation of the liposome from the material for membraneous layer, brings about the improvement of the liposome-yield, and after forming the liposome, brings about the easiness in the process of separating the liposome, the uniformity of the particle size of the liposome and the improvement of pliability, the strength of the wall membrane of the liposome and the slow-releasing property of the active substance included in the vesicle of the liposome when administered within the living body.

The active substance, particularly the physiologically active substance used on the formation of the liposome of the invention is a medicine of which the side effect is to be reduced or a medicine of which the persistency within the living body is to be improved, and is exemplified by peptide hormons such as insuline, oxytocin, vasopression, adrenal-cortex stimulating hormon (ACTH), luteinizing hormon-releasing hormon (LH-RH), carcitonin, somatostatin, steroid hormons such as progesteron, follicular hormon, adrenal cortex hormon and other hormons such as prostaglandin, adenosine-3',5'-cyclicmonophosphate, anti-tumor agents such as chlorambutyl, streptozocin, methothorexate, 5-fluorouracil, sitosin arabinoside, mitomycin C, breomycin, polysaccharide derivative, antibiotics such as penicillin, cepharospolin, streptomycin, enzymatic preparations such as aminoglucosidase, invertase, etc.

The liposome according to the present invention comprises particles of 0.01 to 10 microns in average diameter with a narrow range of distribution of particle diameter, and those particles of 0.5 to 5 microns in average diameter are obtainable easily with a uniform diameter. The particles of liposome of the present invention are excellent in pliability, and they can be concentrated by a low speed-centrifugal treatment at 2,000 to 4,000 r.p.m. Moreover, the re-dispersion after concentration can be carried out only by a short time-shaking without necessitating an ultrasonic treatment to be recovered to the original state.

The liposome according to the present invention, as is

understood from its specific properties described above, is excellent in its capability of maintaining the active substance included within its vesicle as compared to the conventional liposome of the type of pure phospholipid micelle, and also its slow-releasing property to the active substance included therewithin is favorable. Accordingly, the liposome of the present invention is particularly suitable as a protecting agent for an unstable medicine within the living body, and also for a medicine which causes side effects by an inevitable excessive administration. By these reasons, the liposome according to the present invention is possibly utilizable as a medicine.

For instance, in the case where the liposome of the present invention is applied as an insulin injection, an intramuscular administration of the liposome of the present invention once two to seven days at a dose of 0.2 - 20 mg as insulin to an adult gives the same effect as that of the direct intramuscular administration of free insulin three times a day at a single dose of one third of 0.1 to 4.0 mg.

In cases where the liposome of the present invention is utilized as a medicine, its administration is possible via several routes such as percutaneous, subcutaneous, intramuscular, intraperitoneal, intravenal, intrarectal and topical, preferably by subcutaneous or topical administration. The amount of administration depends naturally to the method and the route of administration, the kind of active substance and the extent of treatment, however, usually it is 0.1 to 1 times as large as

the amount of the active substance directly administered per day, and further the interval of administrations can be extended.

In addition, the liposome of the present invention is possibly administered as an injection after dispersing the liposome into an aqueous physiological saline solution. Particularly, the liposome of the present invention containing a peptidic physiologically active substance including peptide hormons exhibits a remarkable effect when applied as a subcutaneous or intramuscular injection. Since peptidic physiologically active substances are generally degraded promptly in the living body, it is necessary to carry out frequent administration (injection) for the maintenance of the effect of the substance resulting not only in causing the heavier load on the patient but also causing the large fluctuation of the concentration of the active substance in blood. These situations are apt to reduce the effect of administration of the active substance and to cause side effects of the active substance.

Whereas, the liposome of the present invention, as are shown later in Examples, shows an excellent slow release of the included active substance on subcutaneous or intramuscular injection, and accordingly, the times of administration are possibly reduced materially with a maintenance of the uniform concentration of the peptidic physiologically active substance in blood, and accordingly, the effect of the active substance is fully exhibited with the suppression of its side effects.

According to the acute toxicity test of the above-

mentioned materials for forming the above-mentioned liposome of the present invention using rats via two routes of subcutaneous and intravenous injections, no toxic sign was observed on the treated rats until the dose rate reached 1,000 mg/kg concerning every material, the fact showing that the liposome of the present invention is safely applicable as a medicine from the viewpoint of the wall membrane of the liposome.

The followings are the concrete explanation of the present invention while referring to Examples.

Example 1:

A solution containing 100 mg of a commercial crude yolk lecithin (manufactured by Merck Company), 11.6 mg of cholesterol and 2.7 mg of stearylamine in 10 ml of chloroform was placed in a 25-ml round-bottomed flask set on a rotatory evaporator, and by distilling choloroform from the solution while rotating the evaporator at a temperature of 38°C under reduced pressure, a film was formed on the inner wall of the flask. Then, one milliliter of an aqueous 1% by weight solution of adenosine 3',5'-cyclic-monophosphate (hereinafter abbreviated as C-AMP) was added to the flask, and by shaking the flask for 30 minutes the film was exfoliated from the inner wall of the flask and the film was dispersed into the solution. By treating the dispersion with a supersonic treating machine (manufactured by Nippon Seiki Company, Model NS 200-2) for 20 min, a suspension-dispersion of particles of 1 to 2 microns in average particle

diameter was obtained. In the next step, an aqueous physiological saline solution of 6 times by volume of the above-mentioned suspension-dispersion was added to the suspension-dispersion, and the mixture was treated 3 times with centrifugal separation at 3,000 r.p.m. for each 10 min to separate completely the thus formed liposome, 1 - 1 and the residual solution of C-AMP which had not uptaken into the liposome. For comparison, four kinds of liposomes were prepared by using the materials for membraneous layer shown in Table 1, as Comparative Examples, 1 - 2, 1 - 3, 1 - 4 and 1 - 5 in the same procedures as in 1 - 1.

The rate of entrapment of C-AMP, that is, the percentage by weight of C-AMP collected within the vesicle of the liposome to the amount of C-AMP used, and the extent of release of C-AMP from the liposome after 24 hours of maintaining the liposome at a temperature of 37°C, that is, the residual percentage in the liposome, are shown in Table 1. As is seen in Table 1, by the use of the crude lecithin as the material for the membrane layer, the liposome of improved rate of entrapment and of the improved residual amount in the vesicle of the liposome as compared to those of the liposome prepared by using the conventional material for membraneous layer.

Table 1: Rate of Entrapment and Residual Percentage

Classification	Specimen	Composition of material for membrane (mg)	Rate of entrapment* (%)	Residual percentage of C-AMP within liposome vesicle**
Present invention	1 - 1	crude lecithin(+) 100 cholesterol 11.6 stearylamine 2.7	28	99.2
Comparative Examples	1 - 2	purified lecithin(++) 100 cholesterol 11.6 stearylamine 2.7	1.0	89.0
	1 - 3	purified lecithin 100 stearylamine 2.7	3.4	88.9
	1 - 4	purified lecithin 100 cholesterol 11.6	0.6	84.2
	1 - 5	purified lecithin 100	1.6	84.3

Note: (+) commercial material , (++) Commercial material

* Amount of C-AMP entrapped into the liposome expressed by percentage by weight of the amount of C-AMP used in preparation of the liposome.

** After keeping the liposome at 37°C for 24 hours.

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In addition, commercial crude lecithin (manufactured by Merck Co.) and commercial purified lecithin (manufactured by Sigma Co.) had the following compositions, respectively:

unit: % by weight

Specimen	Phopholipid	Cholesterol	Oily substance
Crude lecithin(+)	93.8	1.1	5.1
Purified lecithin(++)	99.5	0.3	0.2

Example 2:

Liposomes were prepared by the same procedures as in Example 1 except for using one milliliter of an aqueous 20% by weight of glucose solution instead of an aqueous solution of C-AMP in Example 1 using each of the following materials for membraneous layer of the liposome shown in Table 2.

The amount of cotton seed oil as a component of the materials for the membraneous layer and the rate of collecting glucose in each liposome are correlated in Fig. 1. In addition, the residual amount of glucose in the liposome as compared to the initial amount of glucose in the liposome by percentage after maintaining for 24 hours at a temperature of 37°C was shown in Fig. 3, the determination of the above-mentioned glucose being carried out on Specimens of 2 - 7, 2 - 8 and 2 - 9.

From these figures, the superiority of the liposome of the present invention to the conventional liposomes is clearly recognized.

Table 2

Composition of Materials for Membraneous Layer

Classification	Specimen	Composition of Materials for Membrane	
		Base material (mg)	Cotton seed oil (mg)
Present invention	2 - 1	Crude lecithin(+) 100	0
	2 - 2	"	5
	2 - 3	"	10
	2 - 4	"	15
	2 - 5	"	20
Comparative example	2 - 6	"	40
	2 - 7	Purified lecithin(++) 100	0
Present invention	2 - 8	"	5
	2 - 9	"	10
	2 -10	"	15
	2 -11	"	20
Comparative example	2 -12	"	40

Note: (+) commercially available

(++) commercially available

Example 3:

By using the materials for membraneous layer having compositions shown in Table 3, while using the procedures described in Example 1, each film was formed on the inner wall of a round-bottom flask. After adding one milliliter of an aqueous citric acid-buffer solution containing 10 mg of insulin per 10 ml of the solution (pH of 2.3) to the flask, a suspension-dispersion of liposome particles of 1 to 2 microns in diameter was prepared by the same procedures as in Example 1. After leaving the suspension-dispersion at room temperature for 24 hours, it was treated one with 6 ml of an aqueous physiological solution and two times with a 6 : 1 mixture (by volume) of a physiological saline solution and an aqueous citric acid-buffer solution and subjected to centrifugal treatment to obtain a liposome. After adding an aqueous citric acid buffer solution to the thus prepared liposome to adjust the concentration of insulin in the mixture to 40 IU/ml (IU means an International standard unit), the mixture was subjected to the following experiment:

Table 3

Classification	Specimen	Composition of Material for Membraneous Layer			
Comparative Example	3 - 1	Commercial purified lecithin 100 mg	Cholesterol 11.6 mg	Stearylamine 2.7 mg	Cottonseed Oil 0 mg
Present Invention	3 - 2	as above	as above	as above	Cottonseed Oil 6 mg
do	3 - 3	as above	as above	as above	Cottonseed Oil 12 mg
do	3 - 4	Commercial crude lecithin 100 mg	as above	as above	Cottonseed Oil 0 mg

Experiment: Persistency test of insulin liposome within living bodies

Four groups of SD female rats artificially attacked by diabetes with the administration of streptozocine were respectively given subcutaneous injection of each liposome prepared as above, and their blood sugar was determined before and after the injection of the liposome. Fig. 4 shows the values of blood sugar with the lapse of time, the ordinate showing the percentage of the concentration of glucose in the blood after injection to the concentration of glucose in the blood before injection, and the abscissa showing the days after injection.

As is seen in Fig. 4, the persistency of insulin in the rat body (in other words, the fact that the reduced value of glucose was maintained for a long period of time) due to the application of the liposome of the present invention prepared by using crude lecithin inherently containing oily substance is far superior to that due to the application of the conventional liposome prepared by using purified lecithin not containing the oily substance.

Example 4:

The present example shows the transition of the active substance which has been included in the vesicle of the liposome after administration into the living body. For that purpose, the following experiments were carried out using a liposome including the tritium-labelled luteinizing hormon-releasing

hormon (LH-RH) as the active substance. The liposome was prepared by the following procedures:

Preparation of liposome:

The same film consisting of crude yolk-lecithin, chlosterol and stearylamine was prepared on the inner wall of a round-bottomed flask as in the first paragraph of Example 1. To the flask, 1 ml of an aqueous physiological saline solution containing tritium-labelled LH-RH (hereinafter referred to as ^{3}H-LH-RH) (250 μ Ci/7.3 μg, prepared by New England Nuclear Company) at a rate of 1 μg/ml was added, and after exfoliating and dispersing the film from the inner wall of the flask by shaking the flask for 30 min, the thus formed dispersion was treated by the supersonic treating machine (refer to Example 1) for 15 min to prepare a supension-dispersion of particles of 1 to 2 μ in average particle diameter. Six times by volume of an aqueous physiological saline solution as much as the suspension-dispersion was added to the suspension-dispersion and by subjecting the mixture to 2 times of centrifugal separation at 3,000 r.p.m. for each 10 min, the thus prepared liposome was completely separated from the solution of ^{3}H-LH-RH which has not been taken into the vesicle of the liposome.

The rate of collecting ^{3}H-LH-RH was 10% by weight. After adjusting the concentration of ^{3}H-LH-RH to 3.42 μCi/ml by the addition of an aqueous physiological saline solution, the solution was used for the following experiments.

Experiment 1:

The thus prepared liposome and the free ^{3}H-LH-RH not included in liposome were respectively injected subcutaneously into each ICR male mouse of body weight of 30 to 32 g of each group consisting of 3 animals at a dose rate of 0.34 μCi/animal, and the content of ^{3}H-LH-RH (expressed by the amount of the radio-isotope, RI) in the animal's blood was traced with the passage of time by collecting each 0.25 ml of blood specimen from each animal at a predetermined time interval after treating the specimen with a sample oxidizer (made by Packard Company) and determining the radioactivity by a liquid scintillation counter.

The results of determination were shown in Fig. 5, by taking the content of ^{3}H-LH-RH in the blood specimen after 15 min of the administration as 100.

As is seen in Fig. 5, the hormon included in the vesicle of the liposome prepared according to the present invention is slowly released within the living body.

Experiment 2:

The above-mentioned liposome and the free ^{3}H-LH-RH were respectively injected subcutaneously to each SD male rat of each group consisting three animals, and the excreted amount of the radio-isotope in urine and feces was traced with the passage of time. The rate of administration was 0.68 μCi/animal, and the excreted amount of the radio-isotope was determined by scintillation counting the specimen after diluting the urine

specimen with distilled water to 100 ml, or drying the feces and oxidizing it.

The results are shown in Fig. 6 by taking the administered amount as 100, and integrating the excreted amount to the time of determination. No radio-isotope was detected in the faces.

From Fig. 6, it will be recognized that the hormon included in the liposome prepared according to the present invention is slowly released from the liposome within the living body.

Experiment 3:

The same liposome as used in Experiments 1 and 2, and the free ^{3}H-LH-RH were respectively injected subcutaneously into each ICR male mouse weighing 30 to 32 g of each group consisting 2 mice, and the residual amount of the radio-isotope at the site of injection was traced with the passage of time after the administration of 0.165 μCi. The determination of the residual amount of the radio-isotope was carried out by taking out the region of injection and after dissolving the region into SOLUENE* (supplied by Packard Company) using scintillation counting. The results are: (1) as is shown in Fig. 7, after injecting the free ^{3}H-LH-RH, the residual amount of RI decreased remarkably in a short time after injection, whereas (2) as is shown in Fig. 8, it showed an extremely slow reduction when administered in the state of inclusion in the vesicle of the liposome.

According to the results of the above-mentioned

*Trade Mark

experiments 1 to 3, the slow releasing property of the active substance included in the vesicle of the liposome according to the present invention has been verified.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A liposome comprising a wall membrane, having a structure in which molecules of at least one oily substance are present within a bimolecular layer of phospholipid in an amount of 3 to 20% by weight of said phospholipid, and at least one physiologically active substance included in the vesicle of said liposome.

2. The liposome according to claim 1, wherein said phospholipid is lecithin.

3. The liposome according to claim 1, wherein said oily substance is one or a mixture of at least two oily substances selected from the group consisting of mineral oils, waxes and triglycerides.

4. The liposome according to claim 1, wherein said wall membrane comprises crude lecithin containing at least 3% by weight of at least one oily substance.

5. A composition for forming liposome, comprising a phospholipid, at least one oily substance in an amount of 3 to 20% by weight of said composition and at least one physiologically active substance.

6. The composition according to claim 5, wherein said oily substance is one or a mixture of at least two oily substances selected from the group consisting of mineral oils, waxes and triglycerides.

FIG. 1

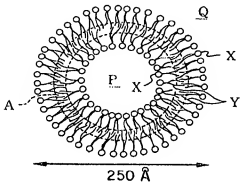

FIG. 2

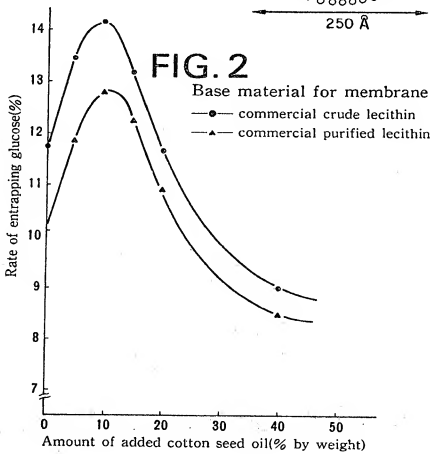

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FIG. 3 Permeability of Glucose

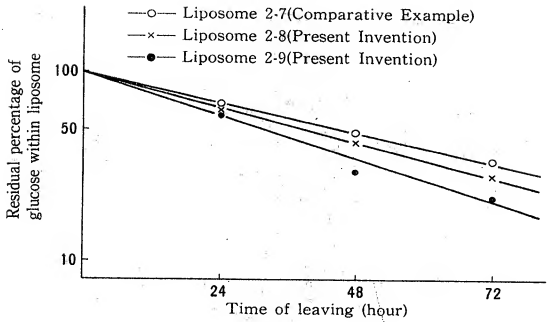

FIG. 4 Persistency of Active Substance (Insulin) within the living body

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FIG. 5

Change of amount of Radio-isotope with the time passage

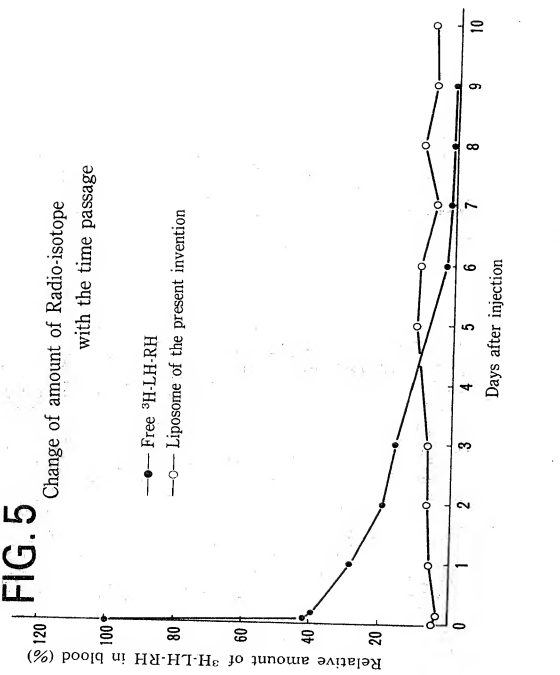

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FIG. 6

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FIG. 7

Residual Amount of Radio-isotope at the Site of Subcutaneous Injection, in the Case Free ^{3}H-LH-RH was injected.

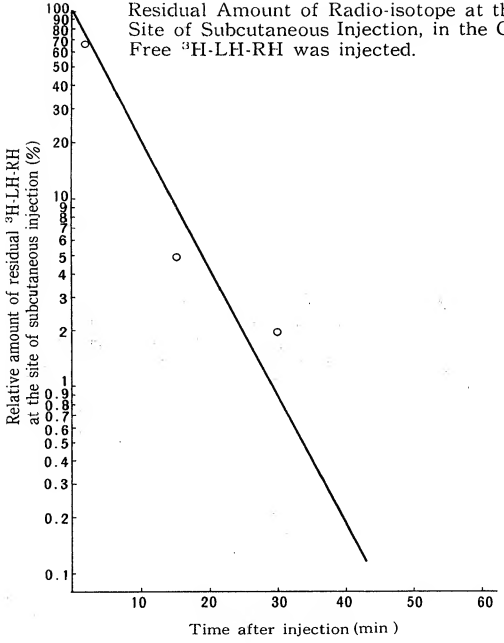
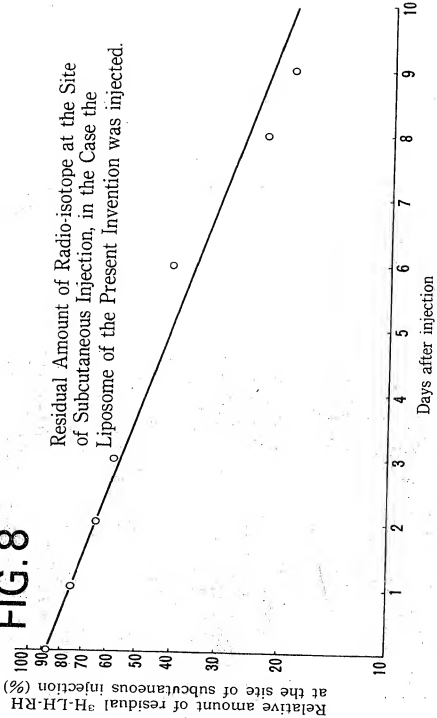


FIG. 8



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